STUDY OF SAPONINS AND SAPOGENINS FROM INDIAN PLANTS

THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN CHEMISTRY

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The work described in this thesis has been carried out in the Department of Chemistry, Aligarh Muslim University, Aligarh (India), under the guidance of Dr. I.P. Varshney, M.Sc.,Ph.D.,(Alig), Dr.ès.Sc.(Paris), F.R.I.C.,(London).

A C K N O W L E D G E M E N T

The author wishes to record his profound sense of gratitude and indebtedness to Dr. I.P.Varshney for his kind supervision and able guidance of this work. His genuine help and unflinching interest in this work was always a source of encouragement and inspiration.

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ABSTRACT

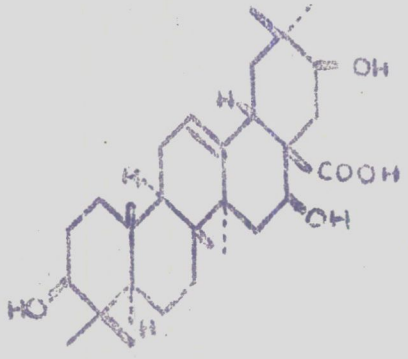
Saponins and sapogenins, broadly divisible into steroidal and triterpenic, occur fairly widely in various parts of the plants. The steroidal sapogenins are important precursors in the synthesis of hormones and therefore much attention has been concentrated on them. Triterpenes which are closely related to steroids are also of significant interest, especially in studies relating to biogenesis, stereochemistry and conformational analysis and have recently been put to use in medicine.

This thesis records the work on saponins and sapogenins from some Indian plants, belonging to the families Leguminoseae, Myrtaceae and Simaroubaceae.

A. Family Leguminoseae:

1. Acacia concinna DC (Shakakai in Hindi): The pods of this plant from Kerala, which are widely used for washing hair in India have been investigated. They have been found to contain a saponin, which on hydrolysis yields a new triterpenic acid, acacic acid m.p. 276-82°; diacetyl lactone m.p. 235-236°; methyl ester m.p. 223-24°; acetyl methyl ester m.p. 203-4°; Bromolactone m.p. 259-62°.

On the basis of degradative studies IR, NMR and Mass (including fragmentational study) spectrographic studies and O.R.D. and Circular dichroism studies, acacic acid has been fixed as 3 β,16 β, 21 β -trihydroxy olean-12-ene-18 β-28-oic acid, with rings A and B in chair form D in quasi-boat form, E in boat form, and with A-B and B-C ring junctions trans fused and D-E ring junction cis fused. Acacic acid therefore can be represented by the following structures.



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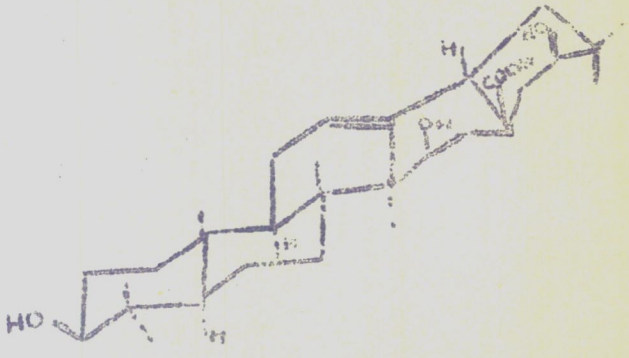

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2. Albizzia amara Benth: Like other species of Albizzia, the seeds of this plant also contain a saponin which on hydrolysis yields a triterpenic acid identified as echinocystic acid.

3. Pithecolobium dulce Benth:(Inga dulcis Willd): The seeds of this plant from Kerala, have been studied and found to contain a mixture of saponins, which on hydrolysis yield two triterpenic acid sapogenins; acetate m.p. 286-88° and 215-22°. The genin, acetate m.p. 286-88° has been identified as proceric acid.

4. Sesbania speciosa Taub: The seeds of this plant from Kerala, yielded a saponin which on hydrolysis furnished a triterpenic acid; identified as oleanolic acid, and β-sitostanol and an unidentified neutral triterpene, m.p. 252-58°, acetate m.p. 162-68°.

B Family Myrtaceae:

5. Psidium guajava Linn: The leaves of this plant from Uttar Pradesh have been investigated and were found to contain a mixture of triterpenic sapogenin identified as crataegolic acid (maslinic acid), guaijavolic acid, in addition to an unidentified acid (methyl ester m.p. 102-4°), β-sitosterol, a hydrocarbon, m.p. 80-82° and a neutral triterpene m.p. 153-55°.

C. Family Simaroubaceae:

6. Balanites roxburghii Planch: The seed kernels of this plant from Uttar Pradesh have been investigated and found to contain a mixture of three saponins, one in major and two in minor quantities. The major one has been obtained in pure form and named as Balanitesin. Balanitesin has been found to be a glycoside of diosgenin contaning glucose, xylose and rhamnose as sugar moities.

This thesis contains the work on the saponins and sapogenins from the above plants, their isolation, characterisation and constitution. The products have been obtained in micro-quantities and the work has been carried out utilising modern techniques such as Thin layer chromatography, Ultra-violet, Infra-red, Nuclear magnetic resonence and Mass spectrography, Optical rotatory dispersion and Circular dichroism.

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INTRODUCTION

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Saponins and sapogenins, broadly divisible into steroidal and triterpenic, occur fairly widely in various parts of the plants. The steroidal sapogenins are important precursors in the synthesis of hormones and therefore much attention has been concentrated on them. Triterpenes which are closely related to steroids are also of significant interest, especially in studies relating to biogenesis, stereochemistry and conformational analysis and have recently been put to use in medicine.

Detailed studies of saponins and sapogenins have been carried out by Noller, Marker, Djerassi, Wall, etc. in U.S.A., Barton, Spring, Halsall, etc. in England, Tschesche, Hagedorn etc. in Germany, Sannie, Lapin, Lederer, Ourisson etc. in France, Ruzicka and Jeger in Switzerland and Kitasato in Japan.

In this department, Varshney and collaborators have studied the saponins and sapogenins from Indian plants. This thesis records the work on saponins and sapogenins from some Indian plants, belonging to the families Leguminoseae, Myrtaceae and Simaroubaceae.

A. Family Leguminoseae:

1. Acacia concinna DC: The pods of this plant which are widely used for washing hair in India have been investigated. They have been found to contain a saponin, which on hydrolysis yields a new triterpenic acid, Acacic acid whose constitution, stereochemistry and conformation has been studied and complete structure assigned.

2. Albizzia amara Benth: Like other species of Albizzia, the seeds of this plant also contain a saponin which has been investigated and the sapogenin identified.

3. Pithecolobium dulce Benth: The seeds of this plant have been studied and found to contain a mixture of saponins, which on hydrolysis yield a mixture of sapogenins, one of which has been identified.

4. Sesbania speciosa Taub: The seeds of this plant yielded a saponin which on hydrolysis furnished an acid genin a neutral genin and a sterol; two of which have been identified.

B. Family Myrtaceae:

5. Psidium guajava Linn: The leaves of this plant have been investigated and were found to contain a mixture of triterpenic sapogenins which have been investigated and identified.

C. Family Simaroubaceae:

6. Balanites roxburghii Planch: The seed kernels of this plant have been investigated and found to contain a saponin, whose constitution has been determined.

This thesis contains the work on the saponins and sapogenins from the above plants, their isolation, characterisation and constitution. The products have been obtained in micro-quantities and the work has been carried out utilising modern techniques such as Thin layer chromatography, Ultra-violet, Infra-red, Nuclear magnetic resonance and Mass spectrography, Optical rotatory dispersion and Circular dichroism.

THEORETICAL

THE SAPONINS

Saponins have been defined by Rosenthaler[1] as "the glycosides (or the corresponding uronic acid compounds), the aqueous solution of which foam copiously and whose aglycones, belong to that of 'polyterpenoids or of 'cholanes." Some cardiac glycosides also exhibit the property of foaming, but they are classified separately due to their characteristic heart action.

The term saponin has been traced to many divergent sources; Bucholz (1811)[2], Grothus (1815)[3] and Gmelin (1819)[4]. It is supposed that this term has been derived from the word 'sapo' meaning soap.[5]

The property of the saponin to form stable lather when shaken with water has been made use of in washing hair, body and clothes since time immemorial. Even since the advent of soap, saponins have been made use of in washing delicate fabrics, which are sensitive to alkali. In India, "soap nut" is still a popular house hold detergent in washing gold ornaments and powdered pods of Acacia concinna is widely used for washing hair.

Another property of the saponins which attracted attention is its toxicity to fish. As fish killed by saponin is edible, plants containing saponins are used to catch fish on a commercial basis.

Properties of saponins:

The properties of saponins can be widely classified as under:

1. Physical properties
2. Chemical properties
3. Physiological properties

1. Physical properties:

The saponins are characterised by the property of foaming in water. The presence of organic solvents like alcohol, ether, acetone, chloroform and especially amyl alcohol inhibit foaming.

Saponins dissolve in water, to form a colloidal solution. They do not dialyse or dialyse very slightly. They stabilise fine suspensions. Saponins are soluble in dilute ethyl and methyl alcohols and some extent in hot amyl, butyl and isopropyl alcohols from which they precipitate on cooling. They are usually insoluble in organic solvents like ether, petroleum ether, benzene, acetone, chloroform and carbon tetra chloride. Certain exceptions, of course, have been noticed to these generalisations, for; the Sapindus mukurossi and the beet root saponinsare soluble in ether while that of Styrax japonica is insoluble in water, but soluble in alkali.

The pure saponin is usually isolated as amorphous powder, and are rarely crystalline.[5] The heterosides of Ivy and digitalis[6] are rare examples. The saponins carry many assymmetric centres and hence they are optically active.

2. Chemical properties:

On enzymatic or mineral acid hydrolysis saponins yield one or more molecules of the same or different sugar or their oxidation products and the aglycone known as the genin or the sapogenin. Hydrolysis is usually carried out by heating the saponin solution with mineral acids and some times under high pressure. Use of milder conditions or organic acids give rise to pro-sapogenins, which are incompletely hydrolysed saponins.

Barium and magnesium hydroxides precipitate saponin from their solutions.[4] Ammonium sulphate has also been used to salt out saponins. Similarly neutral and basic lead acetate or lead acetate in presence of Ammonia also precipitate them on which basis, attempts were made to classify saponins.[7,8]

The saponins form addition complexes with 3β hydroxy steroids,[9] which are insoluble in cold ethanol, ether acetone etc. but soluble in boiling ethanol and methanol, acetic acid and pyridine. Thus digitonin is used in the purification of steroids.[10] The complex formed is decomposed by boiling in xylene,[9] which dissolves the sterol and precipitates the saponin. It is also decomposed by pyridine.[11] The

complex is dissolved in pyridine and poured into ether, which dissolves the sterol and precipitates the saponin. Certain saponins form weak complexes which decompose merely on shaking with ether. Digitonin forms 1:1 molecular compounds with substances other than steroids for example with terpene alcohols, phenols and thiophenols.[12]

3. Physiological properties:

Saponin solutions even upto the dilution of 1:50,000 have been observed to cause hemolysis of blood.[3] This is one property of saponins, very rarely shown by any other plant product. Saponins are extremely toxic to fish and this property has been used since ages in catching fish. Matter of fact these two properties are the basis of formulating the hemolytic index[13] and the fish index.[14] The former is defined as the maximum dilution of saponin, which still causes complete hemolysis of defibrinated blood at pH7.48. The fish index is the minimum concentration of saponin which will kill a fish, (usually carp of 2-4cm in length) in one hour.

Saponins accelerate the germination and the growth of seeds in dilute concentration.[15,16] It stimulates the growth of penicillium.[17] The fermentation of sugar is catalysed by saponins,[18] but this effect is hindered by the presence of salts and certain concentrations of saponins adversely affect the process of fermentation.[19]

The Saponins have an irritating effect on the eyes and have a distinct bitter taste. They also induce sneezing. Examples have been cited of the usefulness of oral intake of insulin[20], calcium lactose[21] and glucose[22] mixed with minute quantities of saponin.

Uses of saponins:

Therapeutic uses cited for saponins include expectorants diuretic and anti-syphilitics[5] but the efficacy as regards the latter has been disproved. Glycyrhizic acid the saponin of liquorice root (glycyrhiza glabra) is a well known expectorant used to this day and is also used in the treatment of peptic ulcer. Mowyah meal is used in destroying earth worms in lawns etc. owing to its saponin content.

Saponins have been put to a variety of uses to the benefit of mankind. It has been used in liquid additives in washing windshields[25], in the manufacture of cellular concrete[24], porous anhydrite wicks[26], and refractory material[26]. In photography, it has been put to a large number of uses. It is used as wetting agent[27] in the coating of photographic films. Saponins in low concentrations improve the drying of emulsions on papers[28] and is also used as a spreading agent in preparation of gelatin solution for swabbing and protecting[29] of the silver layer formed by diffusion transfer. Admixture of saponin in the processing solution in photographic products foam, so that only a small quantity of the solution is

required in processing and only the surface portion is wetted by the solution.[30]

Saponins are also incorporated in stain removers[31] animal repellants,[32] dentifrice,[33] solution used in pickling of alloyed steels,[34] and in foam treatment to confine fumigants to soil.[35] It is also reported that saponins are used in the manufacture of accoustic tiles, ceramics, foam fire extinguishers, to produce foam on beverages, in shampoos, liquid soaps, cosmetic preparations and to emulsify oil for fruit tree sprays.

ISOLATION OF SAPONINS

The isolation of a crude specimen of saponin is an easy task but the purification is a difficult process, which has to be tailored to individual cases, according to the nature of the impurities present. Usually the saponins are extracted with dilute alcohols or water and then purified by the particular method chosen.

Several general methods have been suggested for the purification of saponins which have also been used with certain amount of success in individual cases.[8] Kobert's method consists of addition of an excess of neutral lead acetate to the saponin solution to precipitate the lead complex of the saponin which is filtered. The filtrate is treated with ammonia which precipitates the saponin remaining in the solution. Then the precipitates are suspended in methyl alcohol and decomposed by hydrogen sulphide.

This method often leads to products which are impure. Very often, saponins helps the passage of fine particles of lead sulphide through the filter paper, making the procedure very tedious.

Saponin tannate [36] has been precipitated by the treatment of an aqueous solution of saponin with tannic acid followed by neutralisation with sodium hydroxide. The precipitate is suspended in boiling water and treated with zinc oxide which precipitates the tannic acid. The filtrate is then evaporated to dryness and the saponin extracted with methyl alcohol.

Marker and Collaborators [37] have used the method of precipitating the saponin as the cholesterol complex. Wall and collaborators [38] have proposed a general method. The aqueous solution of the saponin adjusted to pH 4-5 and containing 3% sodium chloride is extracted with water saturated butanol and the solvent recovered to give the saponin.

Another method in vogue is to dissolve the saponin in a small quantity of alcohol and precipitate by drop wise addition to large volume of ether or acetone. This process is repeated till a pure sample is obtained.

Another method of the purification consists of the acetylation of the saponin with acetic anhydride and pyridine [39]. The acetate can be further purified by crystallisation or by precipitation by the addition of a chloroformic solution of the acetate to petroleum ether. The saponin is regenerated by suspending the acetate in sodium hydroxide solution of the

appropriate strength to deacetylate the saponin and the alkali neutralised by passing through a column of ion-exchange resin.

The choice of any particular method of purification depends on the nature of the impurities present and very often a combination of two or more methods may be necessary. The homogenity of the saponin is tested very often by paper chromatography and paper electrophoresis. Thin layer chromatography has also been employed for this purpose.

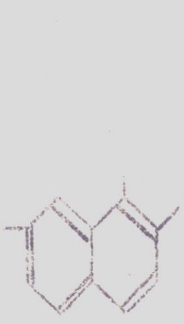
CONSTITUTION OF THE SAPONINS

The saponins on enzymatic or acid hydrolysis yield the sugars and the aglycone known as the sapogenin or genin. The sugars of common occurence are the pentoses such as arabinose, xylose and rhamnose, the methyl pentoses and hexoses such as glucose, fructose and galactose. Units of glucoronic or galacturonic acids are also encountered with. In structural studies the sapogenin is of primary interest.

The initial breakthrough as regards the constitution was achieved by dehydrogenation experiments. Of the various methods of dehydrogenation as zinc, sulphur, selenium and palladised carbon, selenium dehydrogenation was found to lead to better yields and involved lesser side reactions, in spite of the use of higher temperature ($320-50^{0}$). On the basis of these studies saponins were divided into two classes;

1. Triterpenic saponins
2. Steroidal saponins.

The former yield principally sapotalene (1,2,7-trimethyl naphthalene)(I) or 1,8-dimethyl picene (II) and the latter Diel's hydrocarbon (3'methyl 1,2-cyclopentano phenanthrene (III)



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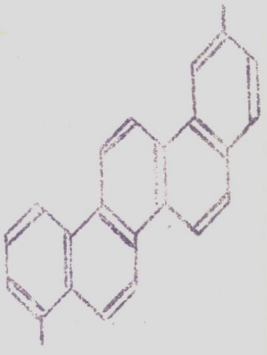

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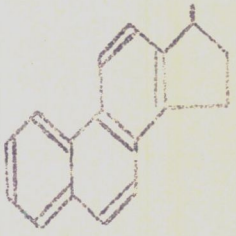


III

Even though the dehydrogenation studies provide a fool proof method of classification of the sapogenins, the large quantity of material necessary, the low yield of hydrocarbons and the difficulty of separating them, makes this method less attractive. Hence the following methods have also been used to differentiate between the two classes of sapogenins.

1. Colour Reactions
2. Molecular Rotation
3. Infra red spectra.

Colour Reactions:

Some of the colour reactions cited below can be carried out on filter paper as well and hence can be used in paper chromatography.

(i) Liebermann or Liebermann Burchard Reaction:

A solution of the substance in cold acetic anhydride is treated with a few drops of sulphuric acid (Liebermann) or the

substance is dissolved in chloroform and treated with acetic anhydride and sulphuric acid. It gives red, violet and blue colours. The triterpenes give green colour directly or through red and blue colours.

(ii) Noller Reaction[40,41]:

0.2 gm. of the substance with 0.5 cc of the reagent (0.01% pure stannic chloride in pure thionyl chloride) is corked in a test tube and left for several hours. A series of shades run through but red persists. Oxyacids containing atleast one free hydroxyl group give a dark positive colouration. This reaction is highly specific for triterpenes.

(iii) Zimmerman Reaction[42]:

An alcoholic solution of a keto steroid with meta dinitrobenzene in caustic potash gives a violet colour and is characteristic of a 3-keto group. 17-keto steroids also answer this test but is negative when the keto group is in 6,7 or 12 portions. Only 3-keto triterpenes give a positive reaction[43]. 1,3-dinitronaphthalene has also been used in which case steroids give a red colour[44].

(iv) Stannic Chloride Reaction[45]:

A small quantity of the substance deposited on a filter paper when sprayed with the reagent (stannic chloride:acetic acid; carbon tetra chloride 60:50:50) and heated in an oven at 100° gives a brown colour. This test is specific for triterpenes.

(v) Sannie's Reaction[46,47]:

The genin deposited on a filter paper, when sprayed with 1 per cent ethanolic cinnamaldehyde solution, dried and resprayed with a mixture of acetic anhydride (12 cc) and sulphuric acid (1 cc) or ethanol, phosphoric acid and perchloric acid (3:5:0.5) develops a yellow colour on heating it indicates the presence of a steroidal genin.

(vi) Para dimethylaminobenzaldehyde Reagent[48]:

A filter paper spotted with the genin is sprayed with a mixture of para dimethylaminobenzaldehyde (0.25 g), ethanol (25 cc) phosphoric acid (4 cc). On heating it produces a brown or yellow colour specific for steroidal genins.

MOLECULAR ROTATION

While the determination of the rotation of the optically active compounds have been practiced, for very many years, it is only within the last ten years or so that it has been realised that such values are a source of much information. Callow and Young[49], first observed, that all naturally occurring sterols having a double bond at the 5:6 position are laevorotatory. They also noted that the 4:5 double bonds increased dextro rotation. The applications of the method were further extended by Wallis and collaborators[50] and finally Barton[51] developed a method by which rotation became an important tool in the elucidation of the structure of steroids and triterpenes. The molecular rotation Md is derived as follows:

$$(M)_D = \frac{(\alpha)_D \times \text{Molecular weight}}{100}$$

The examination of the molecular data of the steroids and triterpenes revealed that there is a well defined and characteristic difference in the molecular rotation values of the two groups of compounds.[52] This difference is attributed to the presence of the gem dimethyl group at C_4 in the triterpenes.

A simple and direct application of this has been the classification of cycloartenol as a triterpene, which was previously beleived to be a steroid.[53] A detailed description of the advantage of molecular rotation will be given elsewhere.

Infra red spectra

The infra red spectra of steroids and triterpenes are characteristic for their respective classes in the 'finger print' region. The spectra of both classes of compounds have been studied in detail and thus infra red spectra can be made use of to differentiate between steroids and triterpenes.

THE TRITERPENIC SAPOGENINS

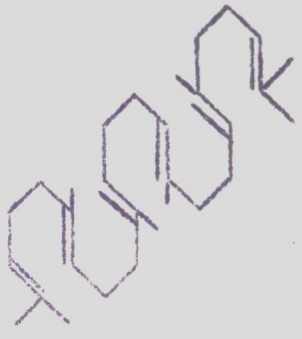
The triterpenes can be classified into four groups on the basis of their carbon skeleton.

1. Acyclic
2. Tricyclic
3. Tetracyclic
4. Pentacyclic

Acyclic triterpenes:

Squalene is the only member of this group. Initially isolated from shark liver oil it has since been isolated from such varied sources as fungii, human ear wax and hair oil.

Tsujimoto[54,55] suggested an acyclic structure for squalene on the basis of the presence of six ethylenic linkages as indicated by catalytic hydrogenation. Heilbron and collaborators[56] showed it to be derivative of isoprene and gave a conclusive proof of its structure (IV). The gross constitution of squalene was proved by Karrer and Helfenstein[57] when they synthesised it.



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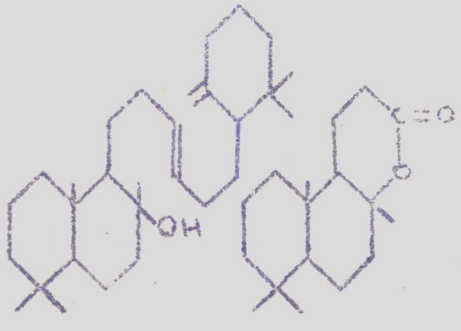
Tricyclic triterpenes:

Ambrein ($C_{30}H_{52}O$) (V) the sole member of this group was first isolated from ambregris[58]. Ruzicka and collaborators[59], by means of extensive experimentations have characterised it as a triterpene alcohol containing two double bonds which are not conjugated and a tertiary hydroxyl group.

Oxidation of ambrein with ozone gave a lactone $C_{17}H_{28}O_2$ (VI) a diketone $C_{12}H_{20}O_2$ (VII) and formic acid; the latter suggesting the presence of an oxomethylene group. The lactone ambreinolide (VI) was found to be identical with the lactone obtained from manool by permanganate oxidation followed by potassium bromite oxidation and lactonisation.[60] Later from amongst the permanganate oxidation products of ambrein a C_{15} hydroxy acid was isolated which was converted to an acid (VIII) earlier obtained from oleanolic acid.[61]

The correlation of these results led to a clear picture of the structure of ambrein (V). Moreover, a relationship between ambrein and diterpenes on one hand and pentacyclic triterpenes on the other hand could be established.



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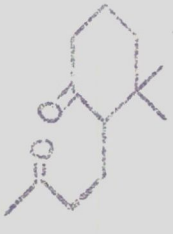

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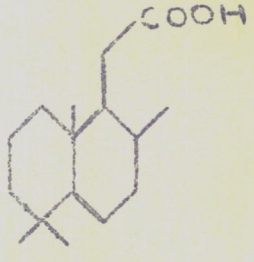
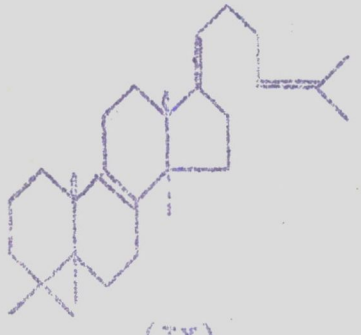


(VII)

(VIII)

THE TETRACYCLIC TRITERPENES

This group consists of several C-30 alcohols and C-31 acids. The two main families in this group of compounds are lanosterol and euphol. Most of the members of this group are structurally similar and the major points of difference lie in their stereochemistry. Thus lano tane (IX) and euphane (X) series differ in the stereochemistry of the fusion of the rings C and D (C-13 and C-14) and in the configuration of the side chain.



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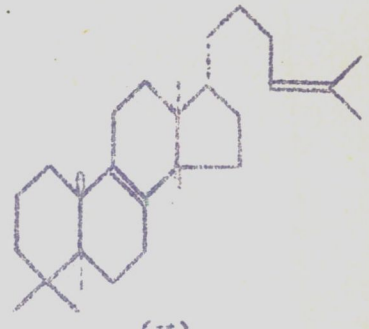
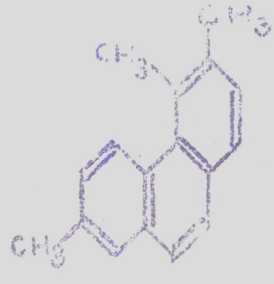


(X)

Lanostadienol (Lanosterol):

Perbenzoic acid titration indicated the presence of two double bonds in lanostadienol[62] and this coupled with the composition $C_{30}H_{50}O$ readily suggested a te-tracyclic formulation. Selenium dehydrogenation of lanostadienol led to 1,2,8-trimethylphenanthrene[63] (XI) as the main product.



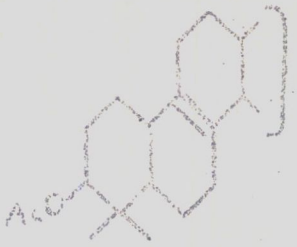
(XI)

The oxygen function was identified as a secondary alcohol by oxidation to a ketone. Further the hydrogenation of lanostadienol[63] hydrogenated only one double bond, thereby indicating that the two double bonds are of unequal reactivity.

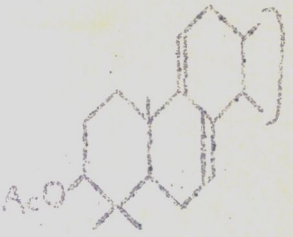
The dehydration of lanostenol with phosphorous penta chloride[65] yielded a doubly unsaturated hydrocarbon, characterised as isolanostdiene (XII). This rearrangement is analogous to that in pentacyclic triterpenes, which carry a hydroxyl group adjacent to the carbon carrying the gem dimethyl group. Thus by analogy, ring A can be pictured as carrying a hydroxyl group at position 3 and a gem dimethyl group at position 4.

(XII)

The infra red spectra suggested the inert double bond to be tetrasubstituted,[66,67] which was further confirmed by the selenium dioxide oxidation of lanostenyl acetate (XIII) to a heteroannular diene (XIV) showing the characteristic ultra violet absorption.[67] The study of the oxidation products of lanostenyl acetate finally fixed the position of the double bond at C 8-9.[66]

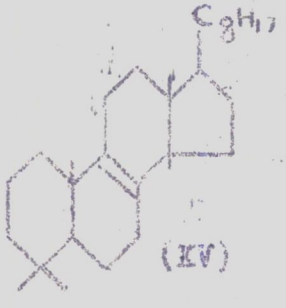


(XIII)

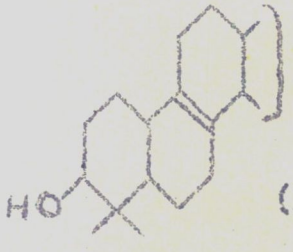


(XIV)

The formation of 1,2,3-trimethyl phenanthrene (XI) by selenium dehydrogenation, coupled with the fact that the hydrocarbon lanosterene (XV) gave a greater yield of 1,2,3-trimethylphenanthrene than did the corresponding alcohol, fixed the positions of the angular methyl groups at C-13 and C-14. These evidences led to a partial structure of lanostadienol (XVI) indicating that the points of attachment of ring D. The size of ring D was proved to be five membered by a study of the ketone formed by the complete removal of the side chain.[69,70]

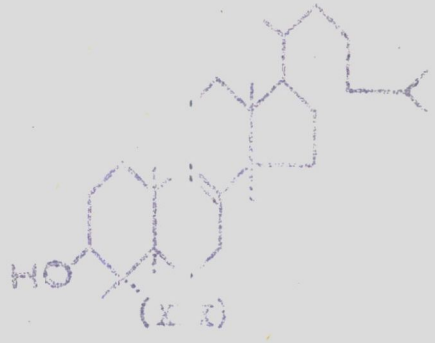


(XV)



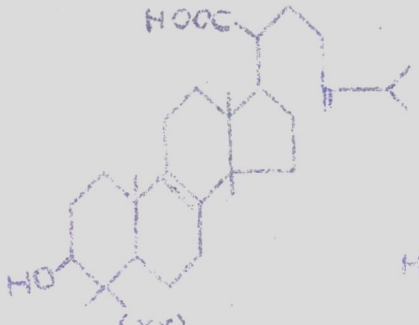
(XVI)

Vigorous oxidation of lanostenyl acetate yielded 6-methyl heptan-2-one as one of the products,[71] showing thereby that the side chain comprised of eight carbon atoms. The isolation of acetone as one of the oxidation products of lanostadienol derivatives carrying the reactive double bond indicated the presence of an isopropylidene group.[72] The final picture of the side chain (XVII) was provided by Ruzicka and collaborators[73]

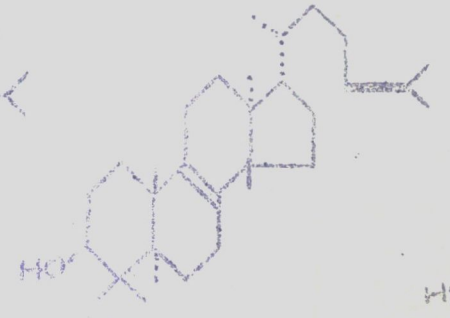


(XIX)

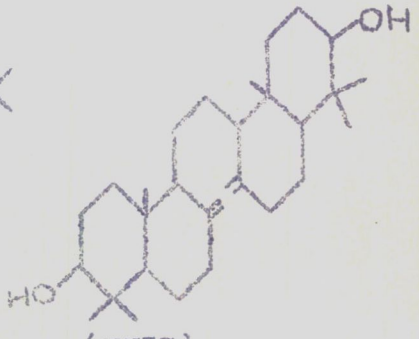
Glauricoic acid a tetracyclic triterpene C-31 acid has the following structure (XX) and euphol which differs from lanostadienol only in the stereochemistry has the structure (XXI). Cnoscarindiol (XXII) is the first tetracyclic triterpene isolated from natural sources, which could be theoretically derivable from a squalene by cyclisation without any rearrangement.



(XX)



(XXI)

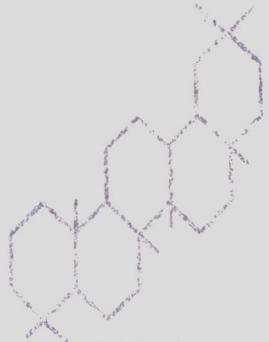


(XXII)

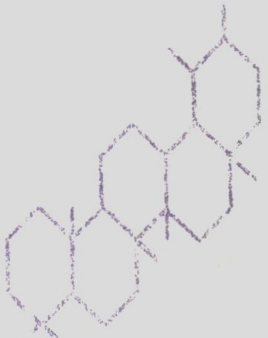
The pentacyclic triterpenes:

The classification of pentacyclic triterpenes is based on three different types of hydrocarbons; (1) Oleanane (XXIII) (2) Ursane (XXIV) and (3) Lupane (XXV) and are accordingly classified to belong to (1) β-amyrin (2) α-amyrin and (3) lupeol respectively. This classification stands good with respect

to the reactivity of the double bond also. The reactivity of the double bond decreases in the order of lupeol group, β-amyrin group and α-amyrin group.



(XXIII)



(XXIV)

(XXV)

To facilitate the classification of a freshly isolated triterpene, certain diagnostic reactions have been devised (Table I)[78] all of which are based on the relative reactivity of the double bond. However, these reactions are not strictly followed in all cases. Thus durorterigenin, a member of the β-amyrin group does not react with selenium di-oxide to form 11,13 (18) diene and the sapogenin E of Styphnodendron coriaceum another member of the β-amyrin group undergoes easy hydrogenation to a saturated compound.[80] Also it was found that α-amyrone oxide is formed by the action of perbenzoic acid on the corresponding olefine.[81]

Further the reliability of the results obtained from the reactions involving N-bromsuccinimide has been questioned[82] and it has been suggested that this reaction is very much dependent on reaction conditions employed. But in general a triterpene belonging to any of these groups will be found to respond to a majority of the specific diagnostic tests listed.

TABLE I

Reagent	Oleanane	Ursene	Lupane
(i) Bromine (on a compound with OOOH at C-17)	12 bromo-lactone	No appreciable bromolactone	Brominated product
(ii) Catalytic hydrogenation	--	--	Double bond reduced
(iii) Cold per-benzoic acid	12,13 oxide which readily isomerises to the 12 ketone	--	20,29 oxide
(iv) Ozone	*	12,13 oxide which readily isomerises to the 12 ketone	29 nor ketone
(v) SeO_2 in acetic acid	11,13(18) diene	-	Unsaturated aldehyde
(vi) N-bromo succinimide	9(11),12,18 triene	9(11),12 diene	**
(vii) Strong acid conditions	Migration of double bond to 13(18) or 18 iso-lactone	--	Expansion of ring E

* Ozone is seldom used in the oleanane series where the milder perbenzoic acid is preffered.

** No sufficient data.

After the carbon skeleton of the pentacyclic triterpenes were determined, work on the different groups of triterpenes led to their structure.

Carbon skeleton of the pentacyclic triterpenes:

The dehydrogenation experiments with selenium have been of great value in the determination of the carbon skeleton of this group of compounds.[83] Pentacyclic triterpenes show a strong tendency to undergo rupture during dehydrogenation and only a minor quantity of aromatic products characteristic of the whole skeleton are formed.

The characteristic and chief dehydrogenation products of pentacyclic triterpenes are the following:

(1) 1,2,3,4-tetra methyl benzene (XXV)

(2) 2,7-dimethyl naphthalene (XXVI)

(3) 1,2,7-trimethyl naphthalene (sapotalene) (XXVII)

(4) 1,2,5,6-tetramethyl naphthalene (XXVIII)

(5) 6-hydroxy-1,2,5-trimethyl naphthalene (XXIX)

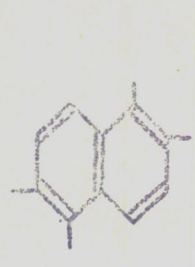
(6) 1,8-dimethyl picene (XXX)

(7) 3-hydroxy-1,8-dimethyl picene (XXXI)

(XXV)

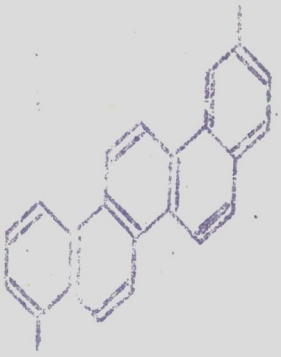
(XXVI)

(XXVII)

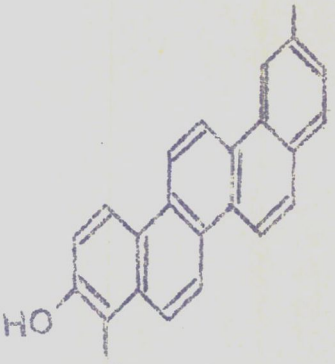


(XXVIII)

(XXIX)

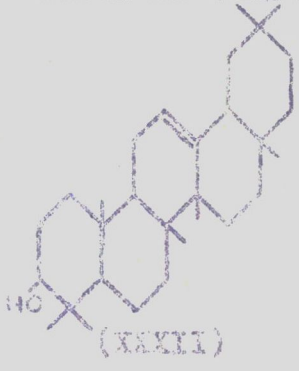


(XXX)



(XXXI)

The origin of these products from β-amyrin (XXXII) can be shown as below:



(XXXII)

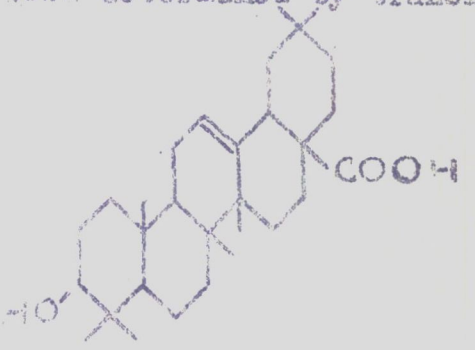
D,E	(XXVI) and (XXVII)
ABCDE	(XXX) and (XXXI)
A,B	(XXVIII) and (XXIX)
A	(XXV)

The formation of (XXV) and (XXVIII) is believed to be due to the migration of one of the methyl groups by a Wagner-Meerwein shift from the geminal position, adjacent to the hydroxyl group at position 3.

The β-amyrin group:

A clear picture of the chemistry of this group was obtained by the reactions on the pivotal compound of this series; oleanolic acid (XXXIII). Initially, the relative positions of the double bond and the carboxyl group in oleanolic

acid was fixed. Further reactions involving oxidative degradation led to the constitution of oleanolic acid. Once the structure oleanolic acid was elucidated, the structure of other compounds were determined by transforming them into known compounds.

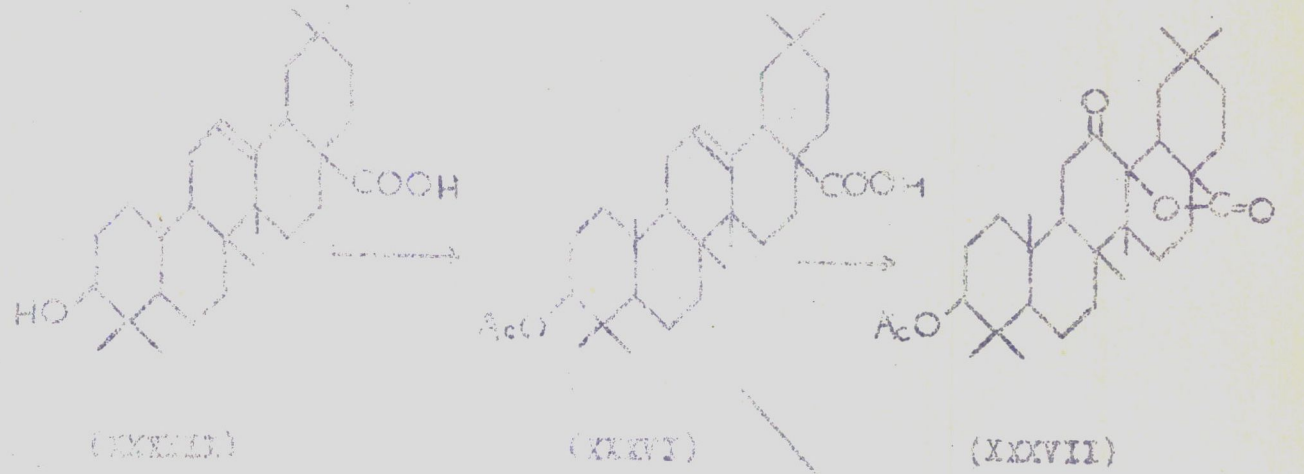


(XXXIX)

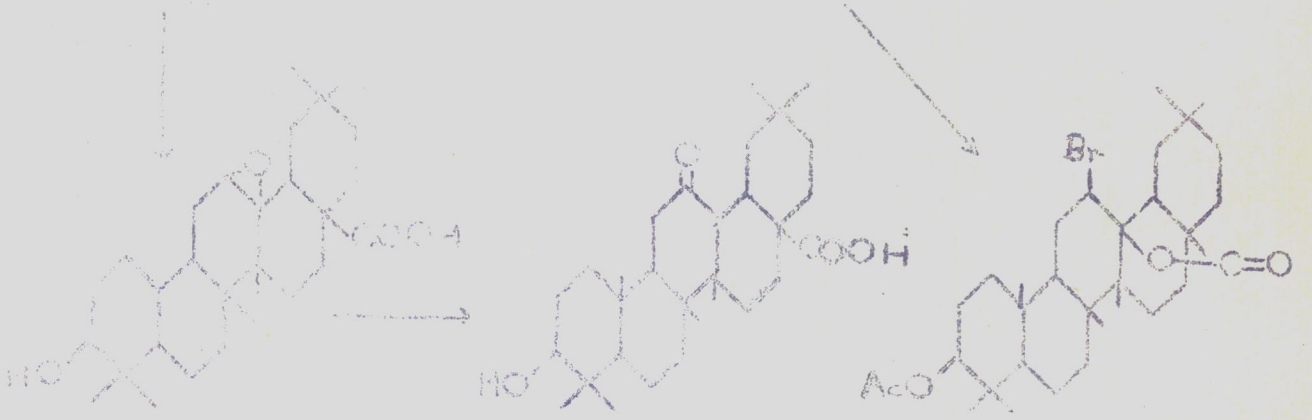
The carboxyl group and the double bond in oleanolic acid:

The monoesterification of an ester by normal acid catalysed esterification and the difficulty in hydrolysing the methyl ester once formed with diazomethane suggested the tertiary nature of the carboxyl group in oleanolic acid.

The presence of a double bond in oleanolic acid could be detected by tetranitromethane and by the formation of an epoxide (XXXIV) which subsequently rearranges to a ketone (XXXV). Oleanolic acid acetate (XXXVI) also gave a ketolactone (XXXVII) and a bromolactone (XXXVIII) in reactions involving the double bond and the carboxyl group.



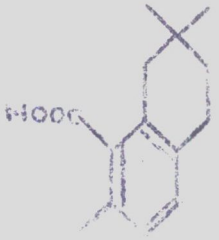
(XXXVI) (XXXVII)



(XXXIV) (XXXV) (XXXVIII)

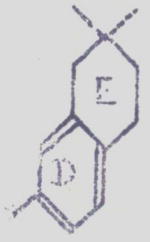
A double bond was located in the ring C at C 12-13 by Ruzicka and collaborators [24-27] by a series of reactions involving the fission of ring C.

The chromic acid oxidation of acetyl oleanolic acid (XXXVI) gave a dihydroxy carboxylic acid (XXXIX) which on further oxidation yielded progressively a hydroxylactone (XL), a ketolactone (XLI) and finally a lactone dicarboxylic acid (XLII). The monomethyl ester (XLIV) of the derived ketodicarboxylic acid (XLIII) was pyrolysed to yield a ketone (XLV)



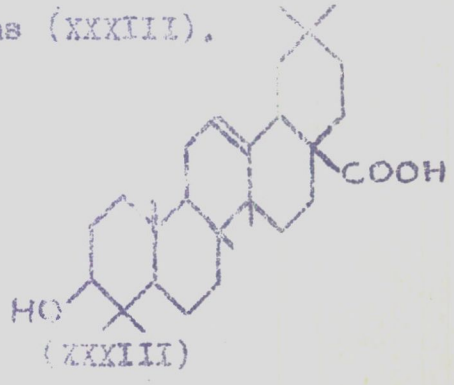
(LIII)

(L)



(LIV)

These reactions clearly showed that the double bond was present in ring C. The formation of a bromolactone (XXXVIII) and a ketolactone (XXXVII) described earlier [88,89] confirmes the double bond to a $\beta\gamma$ or a $\gamma\delta$ position with respect to the carboxyl group. $\beta\gamma$ unsaturated acids undergo easy decarboxylation on pyrolysis with a shift of the double bond to the corresponding $\alpha\beta$ position, and the absence of this type of decarboxylation indicated that double bond was $\gamma\delta$ to the carboxyl at C 12-13. On the basis of these studies oleanolic acid can be written as (XXXIII).



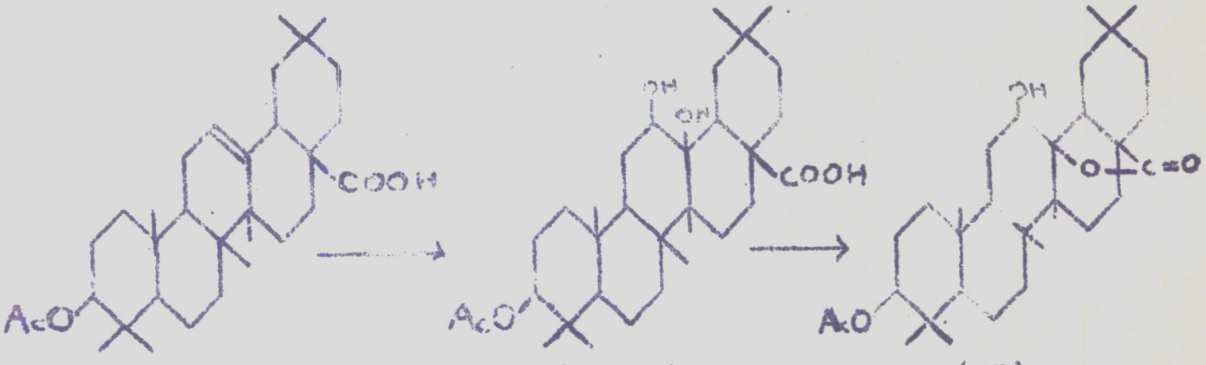
(XXXIII)

Interconversions:

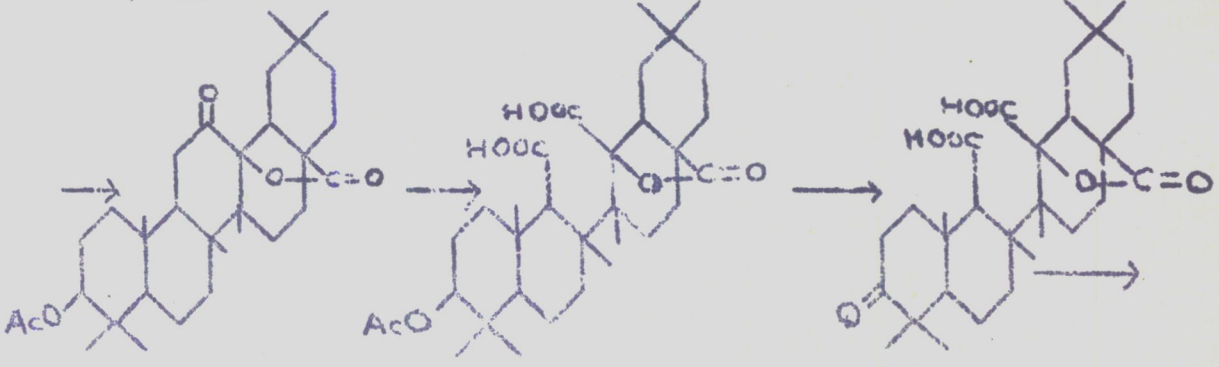
Once the basic skeleton of this group of compounds was conclusively established and the constitution of several compounds established by degradation, the structures of further

and an ester (XLVI). Reduction of the ketone (XLV) to a hydrocarbon (XLVII) followed by selenium dehydrogenation gave 1,6-dimethyl naphthalene (XLVIII).

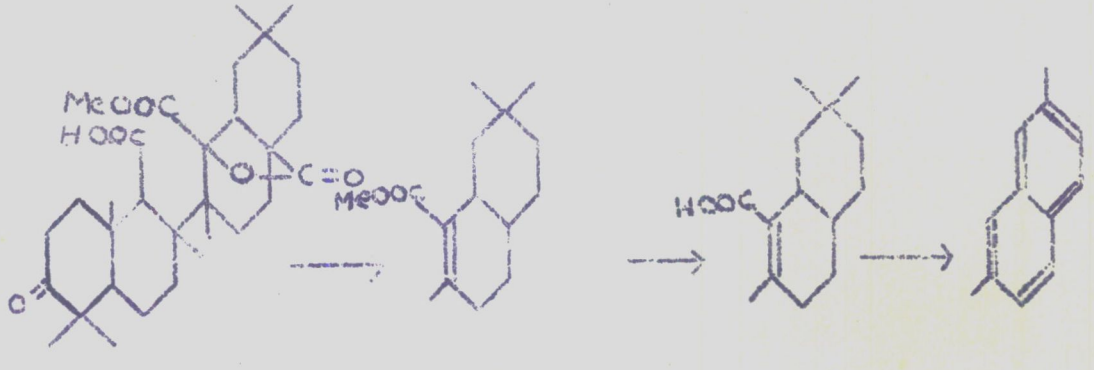
The ester (XLVI) was hydrolysed to the acid (XLIX) and the acid subjected to selenium dehydrogenation to yield 2,7-dimethyl naphthalene (L).



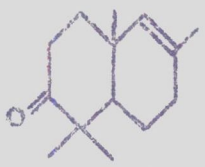
(XXXVI) (XXXIX) (XL)



(XLI) (XLII) (XLIII)



(XLIV) (XLVI) (XLIX) (L)



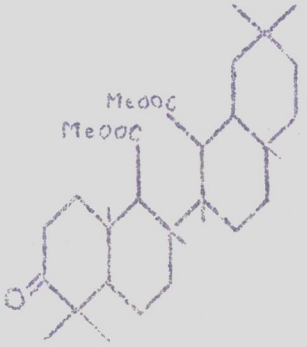
(XLV)

(XLVII)

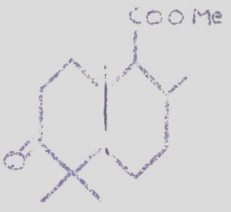
(XLVIII)

The dimethyl ester (LI) of the ketodicarboxylic acid (XLIII) also was pyrolysed to give two fractions; ketonic and ester. The ketone (LII) on reduction gave an ester (LIII), a substance also obtained by the degradation of ambrein.[61] This rigidly established the structure of rings A and B of oleanolic acid.

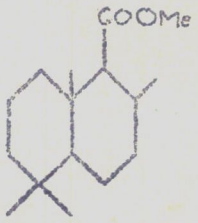
The nonketonic fraction considered to be a mixture of esters was hydrolysed to the acids. These on selenium dehydrogenation gave 2,7-dimethyl naphthalene (L) and (LIV) the latter confirmed by synthesis. Palladised carbon dehydrogenation of the ester mixture after saponification gave an acid, regarded as (LV) due to the close resemblance of its U.V. spectrum to 2,3,6-trimethyl benzoic acid.



(LI)

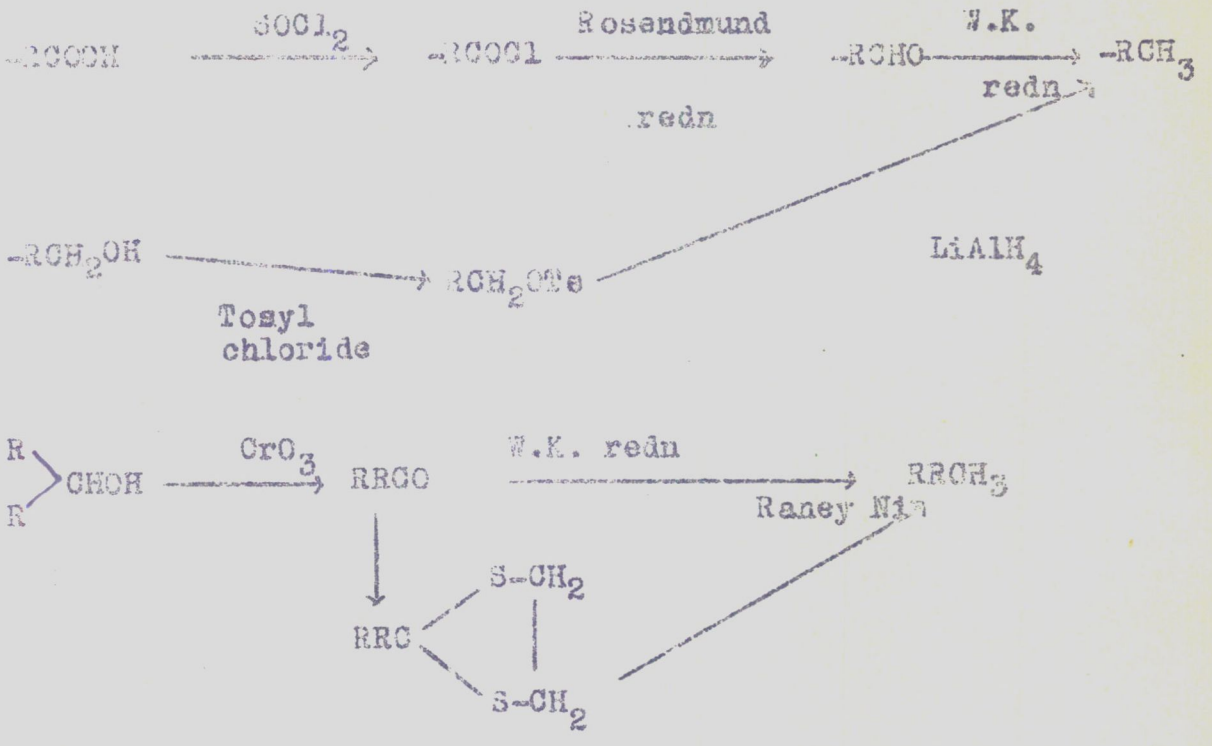


(LV)



(LIII)

compounds could be determined by relating them with known compounds. The usual techniques employed in these conversions are shown below:

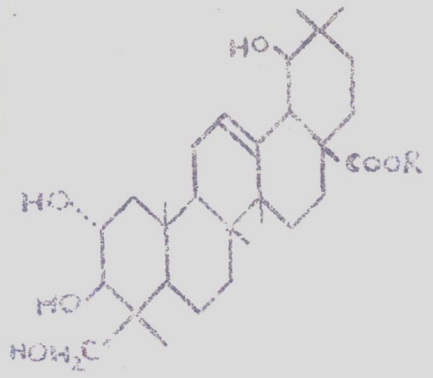


90

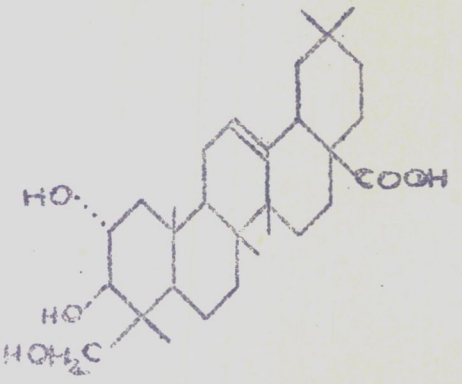
A facile method of converting the CO to CH_2 under mild conditions is by the $NaBH_4$ reduction of the corresponding tosyl hydrazones.

One of the sapogenins recently isolated and studied is tomentosic acid (LVI R = H)[91] obtained from Terminolia tomentosa in which it occurs along with oleanolic acid, arjunolic acid (LVII) and barringtogenol (LVIII). Preliminary studies indicated that it belonged to the β amyrin group.

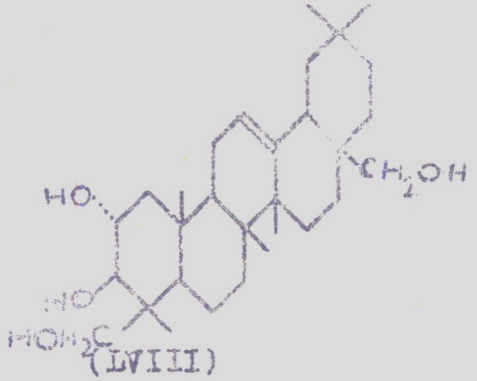
Methyl tomentosate (LVI R = CH_3) and the anhydrolactone (LIX) gave isopropylidene derivatives readily, suggesting the presence of a 1,3-diol system. Copper pyrolysis yielded small amount of formaldehyde which confirmed the presence of a 2,23 diol in the system.[92]



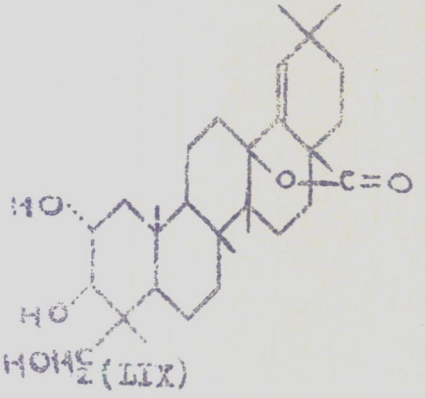
(LVI)



(LVII)



(LVIII)

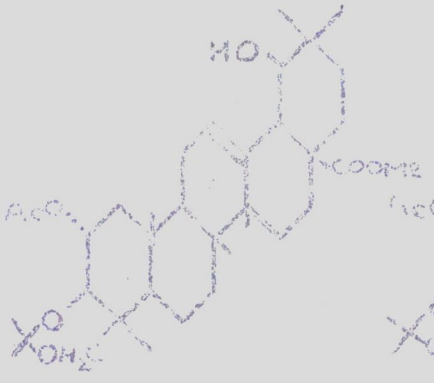


(LIX)

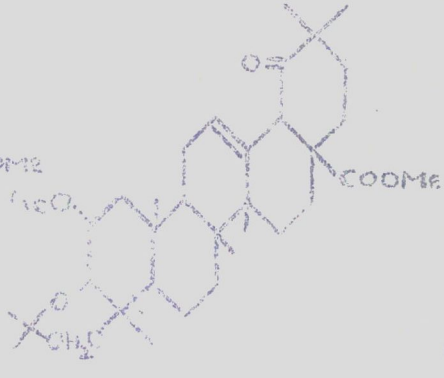
Periodic acid and lead tetra acetate oxidation of methyl tomentosate (LVI R=CH_3) and the anhydrolactone (LIX) require one molar proportion of the either reagent and it was presumed that it contains an $\alpha\beta$ glycol system.[93] The isopropylidene derivatives of both the compounds were inert to lead tetraacetate, which showed that the remaining two hydroxyl groups were not in a glycol system and that one of the hydroxyls is common to the $\alpha\beta$ and to the 3,23 diols. From the foregoing, tomentosic acid must possess a 2α, 3β, 23(24) trihydroxy system as in arjunolic acid (LVII).

Once this relationship was brought to light, attempts were made to relate tomentosic acid to arjunolic acid.

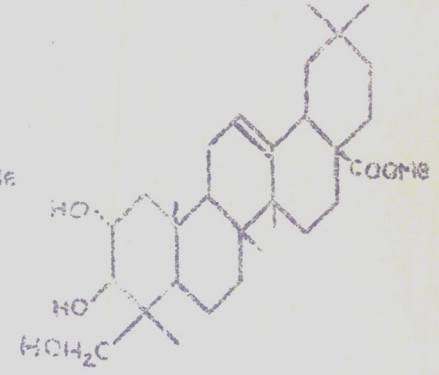
Thus 2 acetyl 3,23 isopropylidene methyl tomentosate (LX) was oxidised to a ketone (LXI), the ketone reduced by forced Wolff Kishner method, the isopropylidene group removed and the resultant product methylated to give methyl arjunolate (LXII).



(LX)



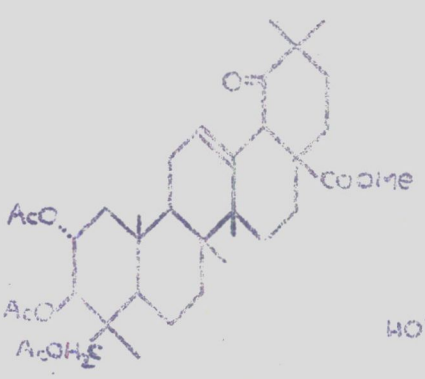
(LXI)



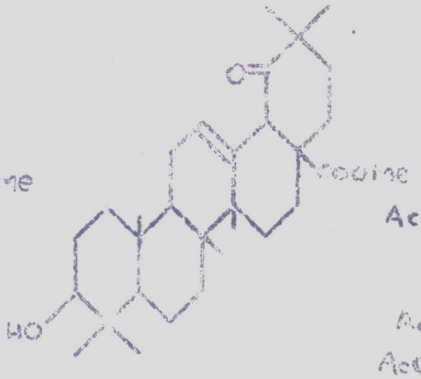
(LXII)

This correlation left the position of one hydroxyl group to be fixed further in tomentosic acid. The ketone (LXI) was inert towards ketonic reagents showing that the keto group is sterically hindered. The ketone also did not give a benzylidene derivative indicating the absence of a methylene group α to the carbonyl.[94]

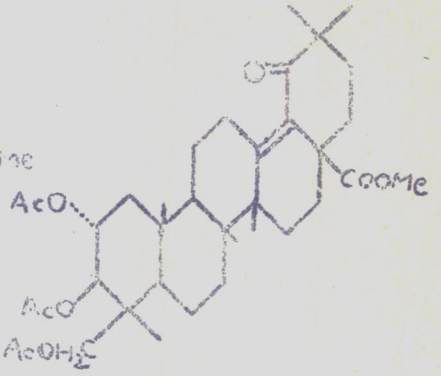
Triacetyl methyl keto tomentosate (LXIII) isomerises in presence of alkali to an $\alpha\beta$ unsaturated ketone (LXIV) (λ max 248 mu) reminiscent of the isomerisation of 19-keto oleanolate (LXV) under identical circumstances.



(LXIII)



(LXV)

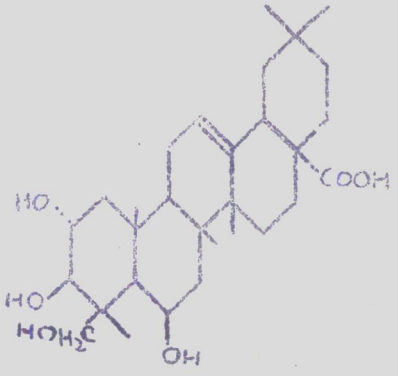


(LXIV)

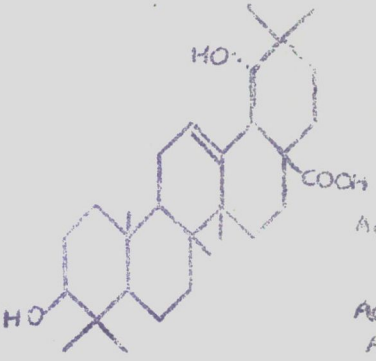
The facile dehydration of tomentosic acid (LVI R = H) to give the anhydrolactone (LIX) could be expected only with hydroxyl groups at positions 6,11, or 19. Tomentosic acid is not an epimer of terminolic acid (LXVI) and because the ketone (LXI) does not form a benzylidene derivative, the hydroxyl group cannot be at positions 6 or 11.

Thus the hydroxyl group should be at position 19,[95] which further relates tomentosic acid to siaresinolic acid (LXVII). If it were so, the 19 OH group should undergo dehydration as in siaresinolic acid to give methyl dehydro arjunolate(LXVIII).[96] $POCl_3$/pyridine dehydration of triacetylmethyl tomentosate (LXIX) did not give (LXVIII). It on the other hand gave an isomeric compound containing an easily reducible double bond, not conjugated with the double bond at C 12-13 (λ max. 216 mμ)

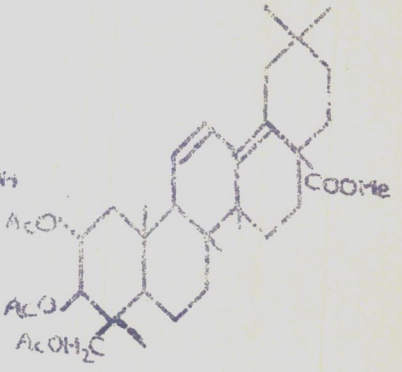
However, the treatment of triacetylmethyltomentosate (LXIX) with selenium dioxide in acetic acid led to (LXVIII). Similar dehydration of the gentol tetraacetate (LXX) yielded



(LXVI)

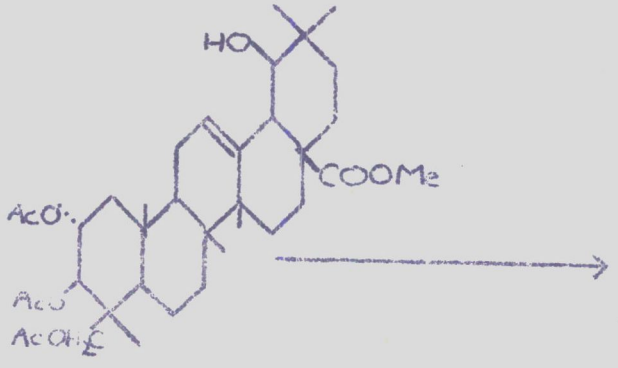


(LXVII)

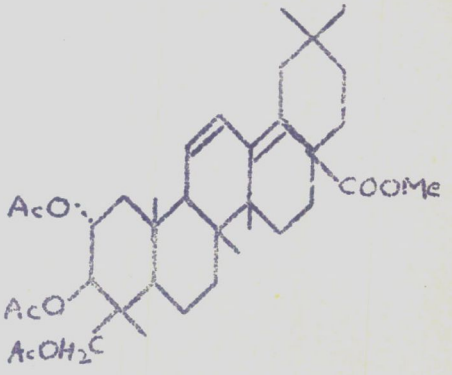


(LXVIII)

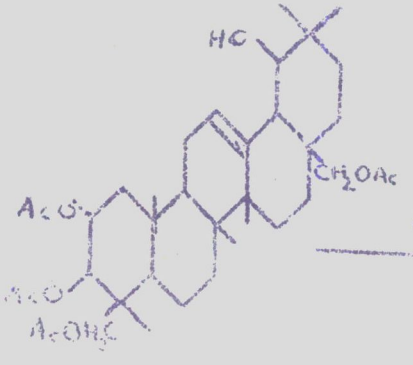
tetraacetyl dehydrobarringtogenol (LXXI) also obtained by selenium dioxide dehydrogenation of barringtogenol tetra-acetate (LXXII).



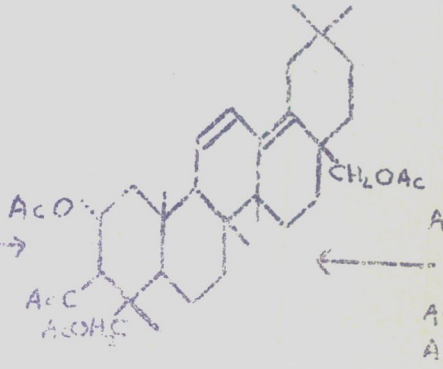
(LXIX)



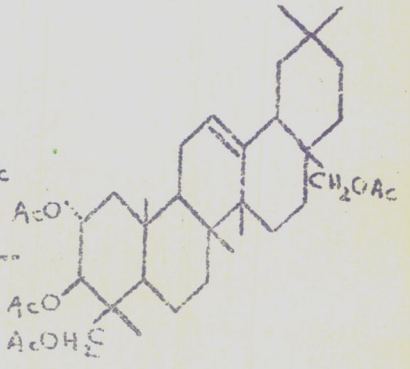
(LXVIII)



(LXX)



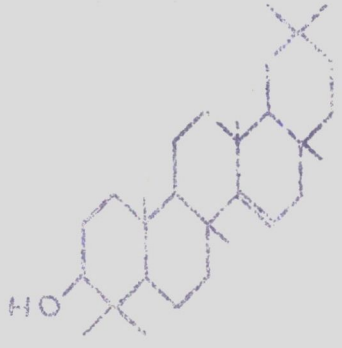
(LXXI)



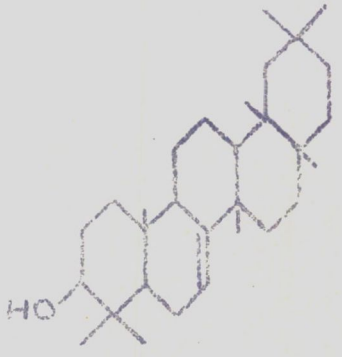
(LXXII)

The formation of dehydro arjunolate and dehydro barringtogenol thus conclusively established the position 19 as the position of the fourth hydroxyl group. The failure of triacetyl methyl tomentosate to undergo dehydration to form (LXVIII) indicated that the orientation of the 19 OH is different from that in sieresinolic acid, in which it is axial; trans to the 18β-hydrogen. Therefore the 19 OH in tomentosic acid must have the β equetorial orientation. This leads to the structure (LVI) for tomentosic acid.

A few other compounds are also known which could be included in the βamyrin group on the basis of the structure of the ring E, in spite of the fact that the skeleton differs from βamyrin. Taraxerol (LXXIII)[97] and multiflorenol (LXXIV)[98] are two examples.



(LXXIII)



(LXXIV)

C. AMYRIN GROUP

This group is comparatively small and only a few members are known. On the basis of the close resemblance of α and β amyrins and the formation of identical products on dehydrogenation it was suggested that the two amyrins were stereoisomers.[99] Also molecular rotation studies[100] indicated that

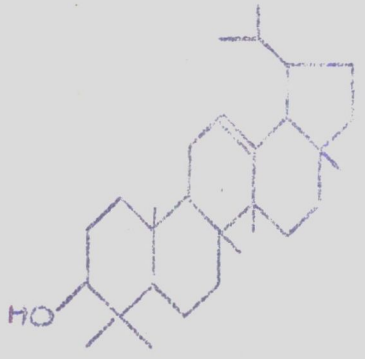
ring A and B were similarly constituted in both α and β amyrins. That the latter assumption was correct and the former wrong was proved by Ruzicka and collaborators by a series of reactions on α amyrin acetate, largely parallel to the reactions on oleanolic acid, described earlier.

α amyrin acetate (LXXV) was converted to the saturated ketone (LXXVI) by the action of ozone followed by acid treatment; or alternatively by the action of perhydrol and acetic acid. Oxidation of the ketone gave a dicarboxylic acid(LXXVII) by fission of the ring). From this the 3-keto dimethyl ester (LXXVIII) was derived and subjected to pyrolysis. The pyrolysis product was separated into a ketonic fraction(LXXIX) and a non-ketonic fraction (LXXX) by Girard reagent T. The ketonic fraction (LXXIX) on subsequent treatment gave an ester (LXXXI) identical with that obtained by the degradation of oleanolic acid. This proved that rings A and B are similar in α and β amyrins.

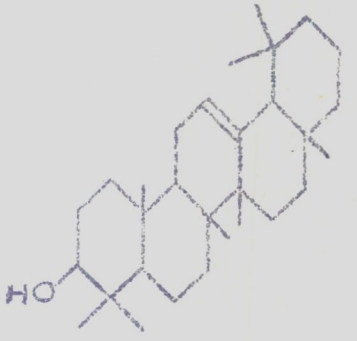
The non-ketonic fraction (LXXX) was subjected to selenium dehydrogenation to yield picene (1,8,7 tri methyl naphthalene (LXXXII). Since the equivalent product from β amyrin was 2,7 dimethyl naphthalene, this suggested that α and β amyrins differ in the position of one methyl group in ring E. On the basis of the above evidence α amyrin can be written as (LXXXIII) a structure, which also explains the more inert nature of the double bond compared, with β amyrin, because of the shielding effect of the 29 methyl group.

103

Infra red studies by Heilbron of the trisnorhydrocarbons derived from the two amyrins and lupeol indicated the presence of two gem-dimethyl groups in α-amyrin, which supported the modified formula (LXXXIV). Further he suggested another formulation for α-amyrin (LXXXV).

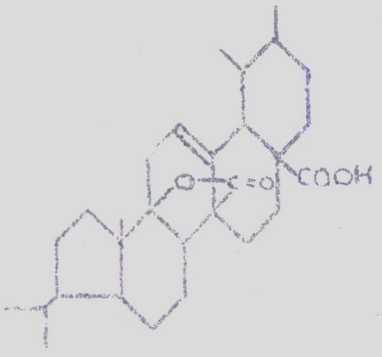


(LXXXIV)

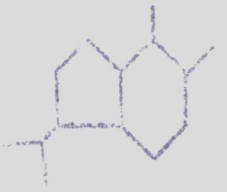


(LXXXV)

The formula (LXXXIV) calls forth for an expansion of ring E during dehydrogenation to account for the dehydrogenation products. That this is not the case was established by a study of a model substance novic acid [104,105] (LXXXVI) identical in rings D and E with α-amyrin. Fission of ring C of novic acid followed by pyrolysis and further treatment led to (LXXXVII) from rings A and B and to (LXXXVIII) from rings D and E which could be explained only on the basis of the old formula (LXXXIII) for α-amyrin. Moreover a detailed study of the infra red spectra of the penta cylic triterpenes in the methyl bending absorption region led Cole and collaborators [106]



(LXXXVI)



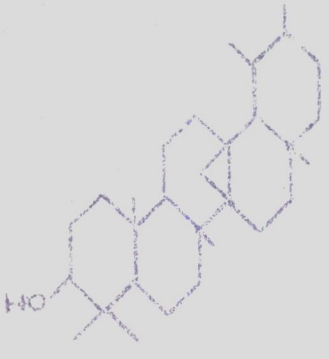
(LXXXVII)

(LXXXVIII)

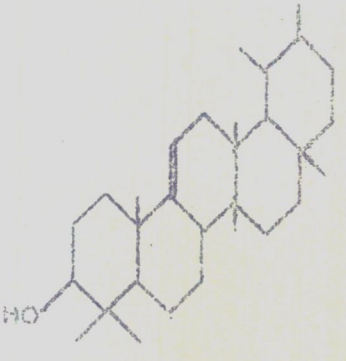
to support the earlier formulation (LXXXVII). The N.M.R. spectrum also supports the earlier formula, originally proposed by Ruzicka.[107] Further this structure was confirmed by the synthesis of αamyrin acetate from methyl glycerritate; a βamyrin derivative.[108]

An interesting member of this group is phyllanthol (LXXXIX) which carries a cyclopropane ring and which undergoes acid induced isomerisation to αamyrin.[109,110]

Arborinol,[111] isolated from the leaves of Glycosmis arborea has been shown to have a carbon skeleton slightly different from αamyrin, in the disposition of one of the angular methyl groups (XC).



(LXXXIX)



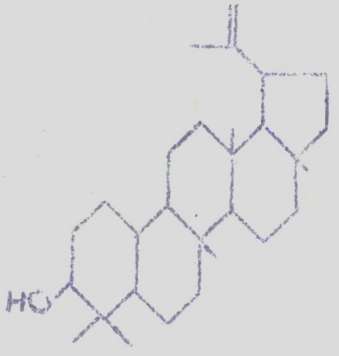
(XC)

LUPEOL GROUP

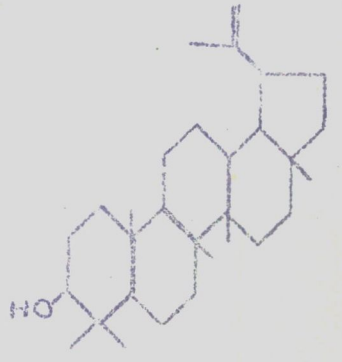
The parent compound of this group is lupeol, first isolated from the seeds of Lupinus albus.[112]

Selenium dehydrogenation of lupeol led to the products originating from the rings A and B only. The absence of picene, 2,7 dimethyl naphthalene and 1,2,7 trimethyl naphthalene indicated that lupeol has a skeleton different from those of both amyrins. Lupeol differed also in that the double bond is easily hydrogenated.[113]

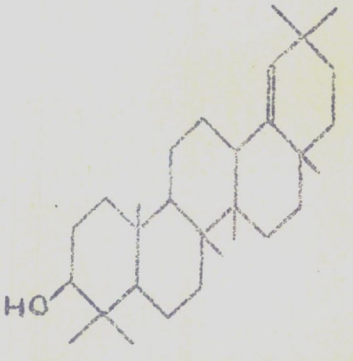
The above reactions coupled with the formation of formaldehyde on ozonolysis led to the proposal (XCI) as representing lupeol.[114] The environment of the isoporpenyl group was shown by the oxidation of lupeol to an $\alpha\beta$ unsaturated aldehyde with SeO_2[115] and by infra red spectra.[116] The structure of lupeol was finally established as (XCII) by the conversion of lupeol into β amyrene III (olean 13(18) ene).[117] This was further confirmed by the conversion of lupeol to germanicol acetate (XCIII) a β amyrin derivative, by the action of acetic anhydride on the hydrochloride of lupeol.[118]



(XCI)

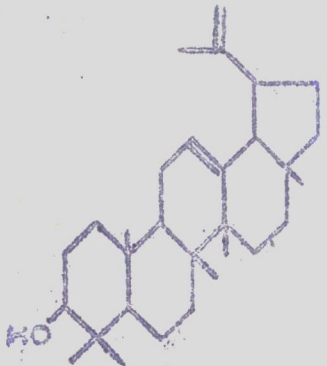


(XCII)

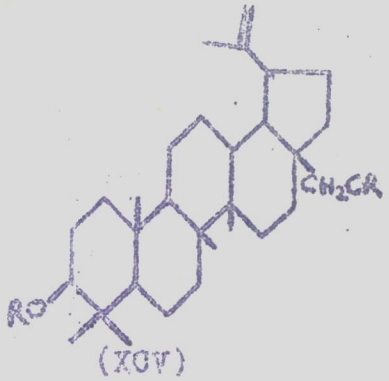


(XCIII)

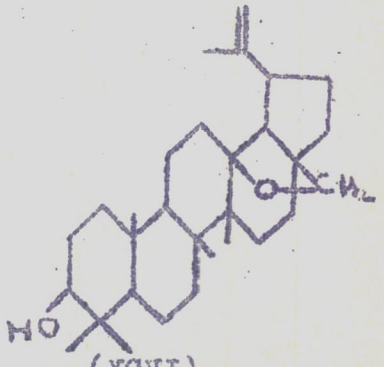
An interesting reaction of lupane group worked out recently is mercuric acetate dehydrogenation.[119,120] They have been found to lead to cyclic ethers or Δ 12,13 compounds. Thus lupeol gives rise to Δ 12,13 (XCIV). Betulin (XCV R = H) yields the 13-28 epoxy derivative (XCVI) whereas betulin diacetate (XCV R Ac) gives rise to Δ 12,13 betulin diacetate (XCVII). Similarly betulinic acid acetate (XCVIII R = H) leads to a lactone (XCIX) whereas acetatemethyl betulinate (XCVIII R = CH_3) leads to a Δ 12,13 derivative (C)



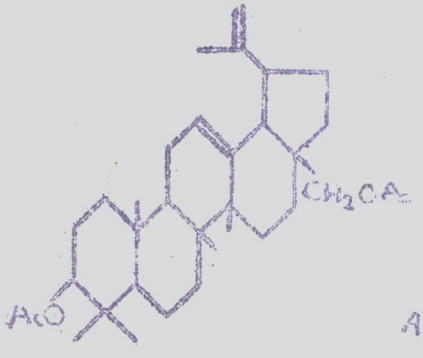
(XCIV)



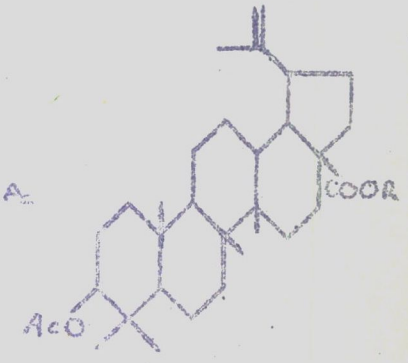
(XCV)



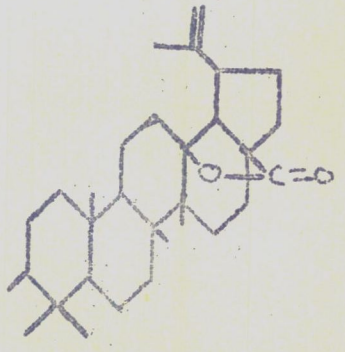
(XCVI)



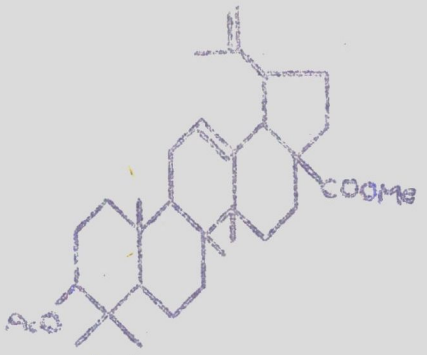
(XCVII)



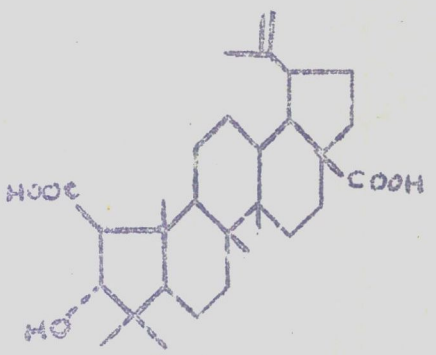
(XCVIII)



(XCIX)



(C)



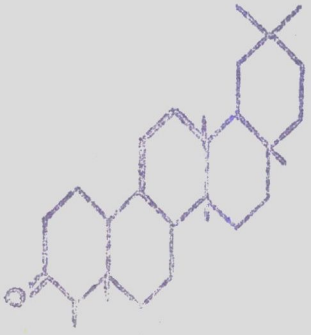
(CI)

This group is the smallest of the three and one of the most interesting compounds belonging to this series is ceanothic acid (CI) an A nor-lupane derivative.[121]

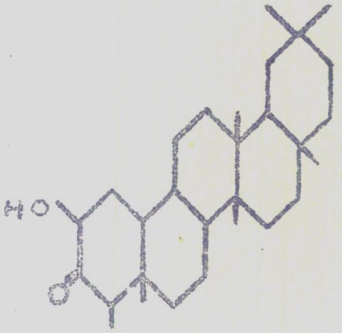
Other Types:

Certain other types of pentacyclic triterpenes are also known and as each type consists of very few numbers a systematic classification becomes difficult.

Friedelin (CII) isolated from cork has been shown to be a triterpane ketone.[122,123] An interesting feature in the structure of friedelin is the absence of the gem dimethyl group at position 4, in contrast with all other types of triterpenes. Cerin (CIII) a ketoalcohol, is another member belonging to this group.

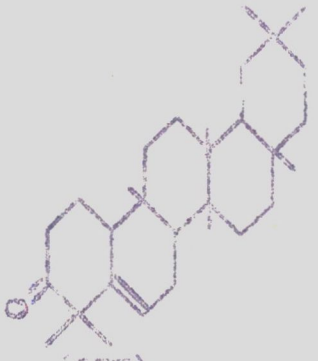


(CII)

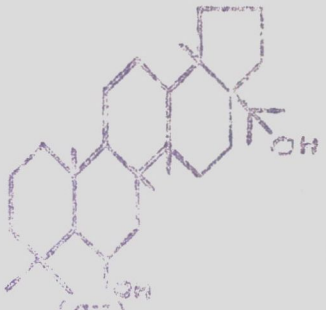


(CIII)

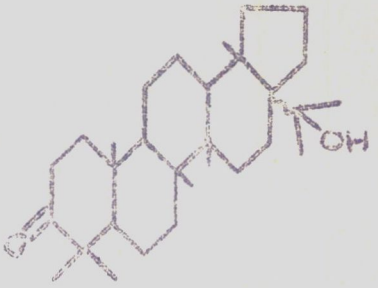
Glutinone or alusenone (CIV) is another type, the structure of which was determined by Spring and collaborators.[124] Zoorin (CV)[125] and hydroxylupenone (CVI)[126] are two other types both having the terminal ring E, five membered.



(CIV)



(CV)



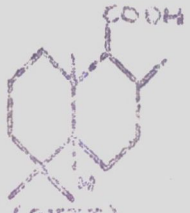
(CVI)

Stereochemistry:

The stereochemistry of the pentacyclic triterpenes can also be considered on the basis of the three groups; β amyrin, α amyrin and lupeol.

β-Amyrin:

The production of the bicyclic carboxylic ester (CVII) from degradation of oleanolic acid derivatives and its correlation with ambrein, manool and abeitic acid established that rings A and B are trans fused.[61] The action of phosphorous pentachloride on oleanolic acid derivatives involving the retropinacolic change further confirmed the part structure (CVIII)

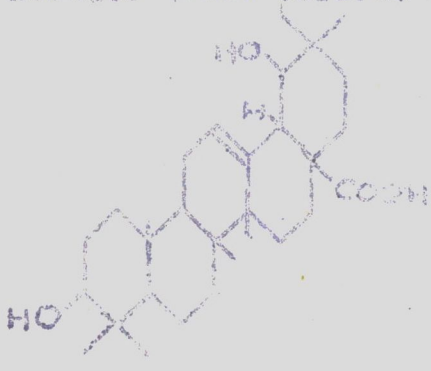


(CVII)

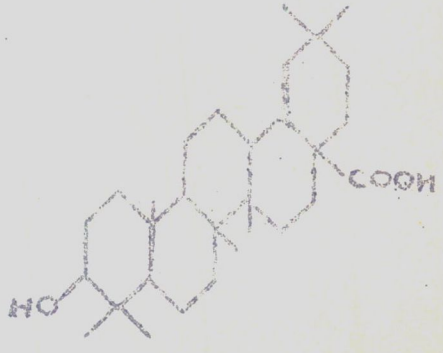
(CVIII)

The next piece of evidence concerns the fusion of rings D and E involving positions 17 and 18. Hirsadiolic acid (CIX) has an axial hydroxyl group at position 18 and as it undergoes easy oxidation, the 18-hydrogen should be trans to it. But as the hydroxyl group and the carboxyl group are both axial and do not undergo lactonization they should be on the opposite sides of the molecule. Therefore, the carboxyl group at position 17 and the hydrogen at position 18 are on same side indicating that the D/E junction is cis in nature.[127]

The relationship of the centers C17 and C18 to the adjoining centers was established by the study of reactions involving morollic acid (CX) and siaresinolic acid (CIX). The diol acetate (CXI) derived from morollic acid has been converted



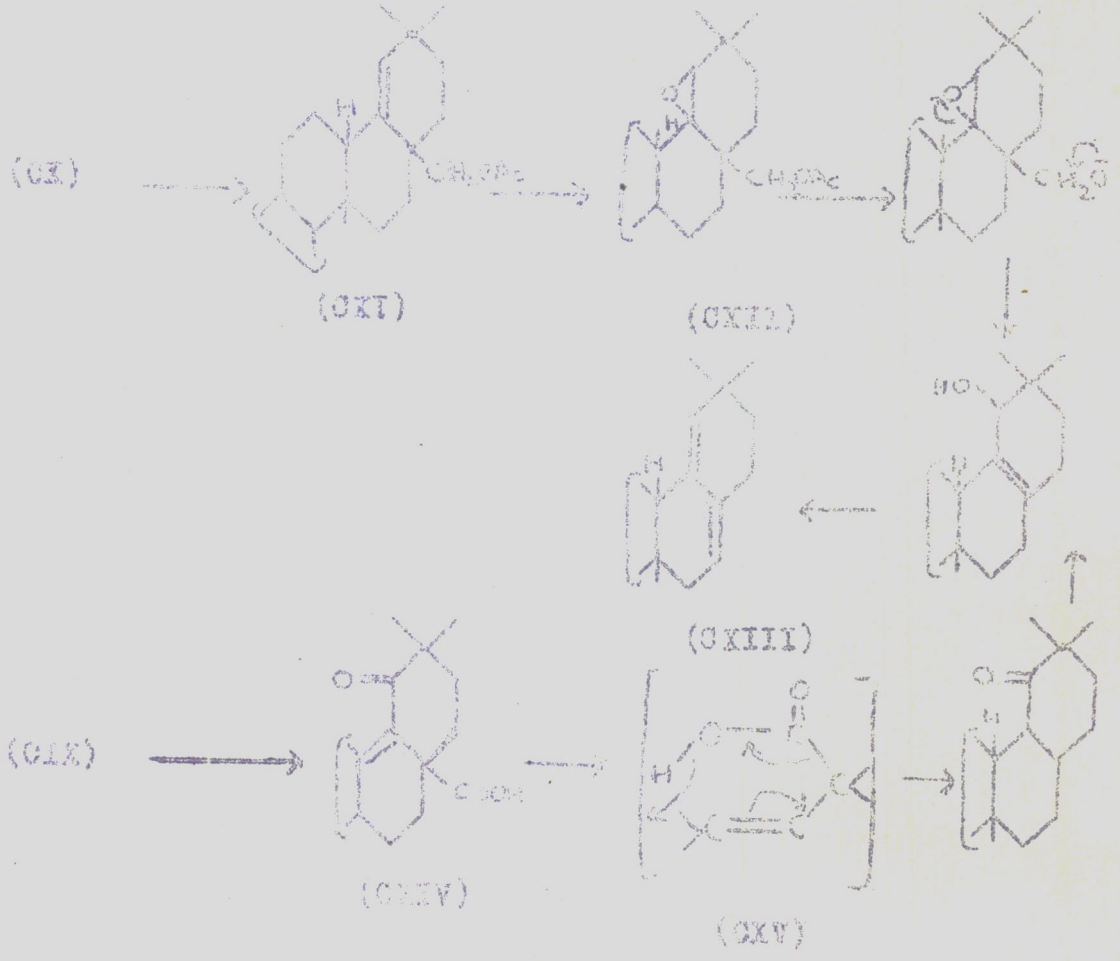
(CIX)

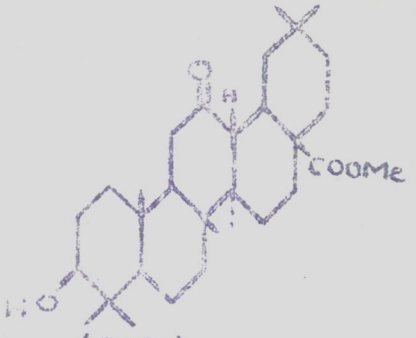


(CX)

to the epoxide (CXII) and further to moroleen 16,18-dienyl acetate (CXIII).[128] In this series of reactions the configuration at C-18 is unaffected and thus should be as in morollic acid. Moroleen 16,18-dienyl acetate (CXIII) has also been obtained from siaresinolic acid. The decarboxylation of

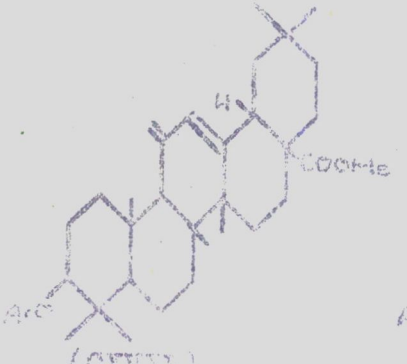
△ 13(18), 19 keto acid (CXIV) undergoes through a cyclic state (CXV) and hence the hydrogen deposited at C-13 should be on the same side of the molecule as was the carbonyl group; i.e. β . Now sitaresinolic acid (CIX) has been converted into morolic acid (CX) through the ketone (CXVI)[129] This conversion involves the treatment of the ketone (CXVI) with strong base and it follows that the configuration at C-13 which is β is stable, and hence the C-14 angular methyl group should be α .



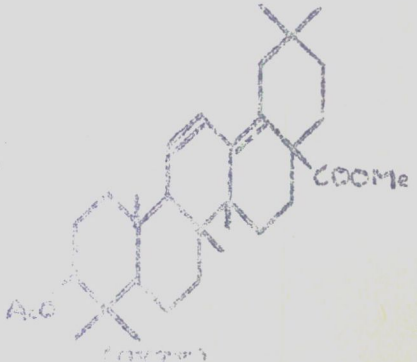


(CXVII)

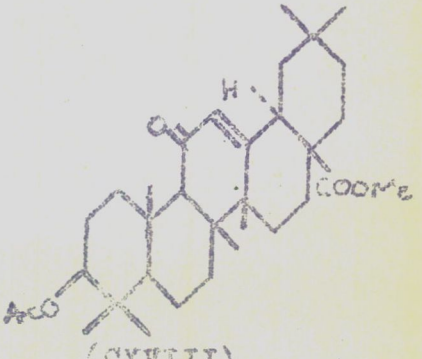
Methyl keto-cleanolate acetate (CXVII) on treatment with alkali gave an isomer (CXVIII). Hydrogenation of (CXVIII) removes the keto group and this product as well as the methyl cleanolate on selenium dioxide oxidation gave the same dehydro derivative (CXIX) which indicated that of the two possible positions, isomerisation took place only at C-18 and not at C-9. Hence it was concluded that the configuration at C-3 was stable and that rings B and C are fused in a stable configuration.[130]



(CXVIII)



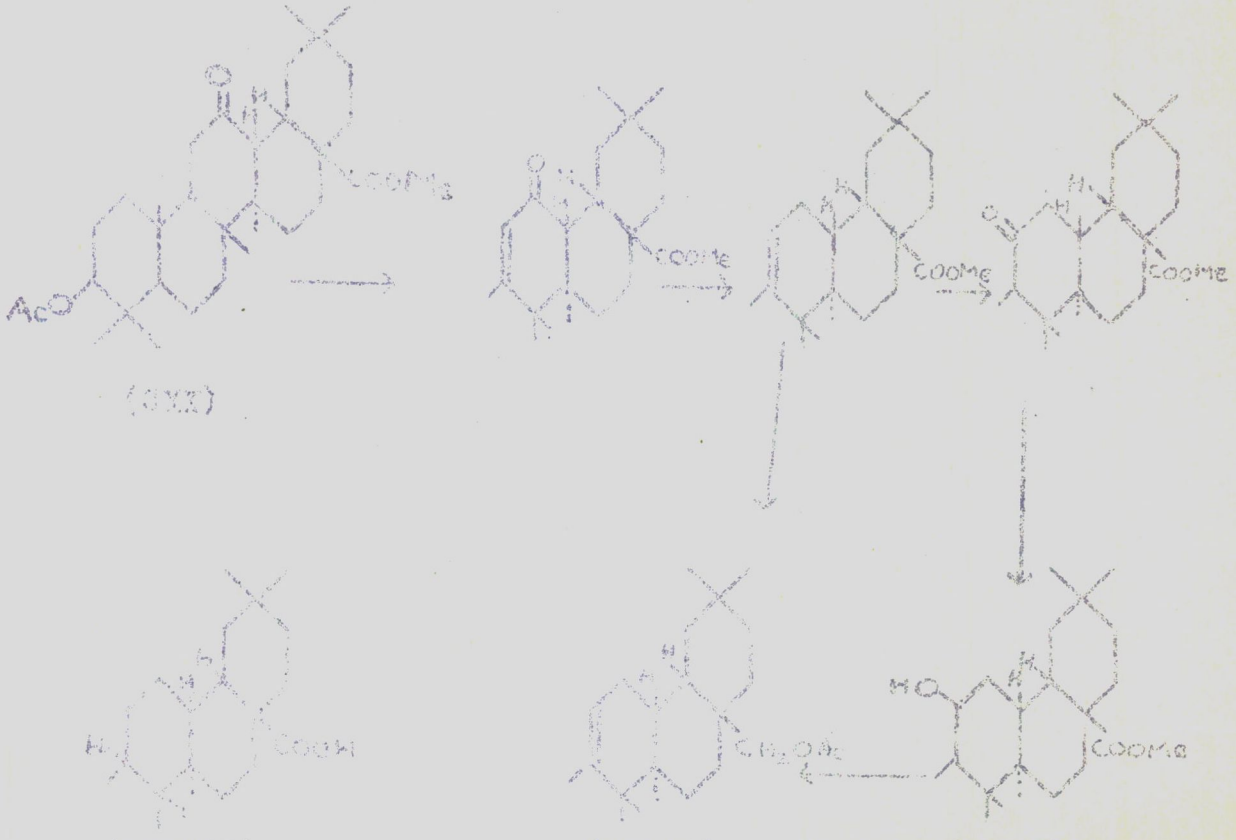
(CXIX)



(CXVIII)

Through the sequence of reactions shown below in which methyl cleanolate (CXX) has been converted into (CXXI) which carries an axial hydroxyl group at C-11. The product (CXXI)

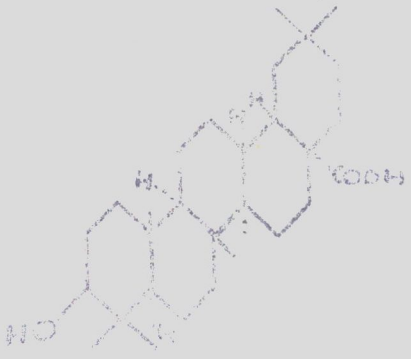
has the 13 hydrogen axial and since the 11 hydroxyl is also axial, the hydroxyl should be β. This axial alcohol (CXXI) undergoes easy elimination to (CXXII) and therefore the hydrogen at C-9 should be α and axial. This suggests the (CXXIII) for oleanolic acid.[130]



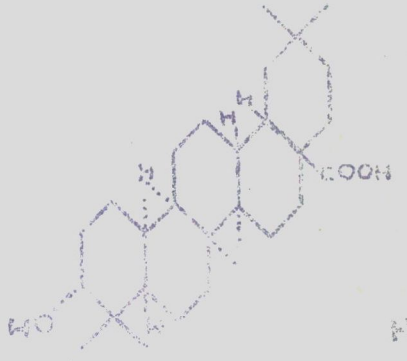
(CXXII) (CXXIII) (CXXI)

Correlation of rings A and B of the molecule with (CXXI) led to two possibilities (CXXV) and (CXXV) which could not be distinguished by chemical means. Molecular rotation arguments supported the formulation (CXXIV).[131] X-ray studies

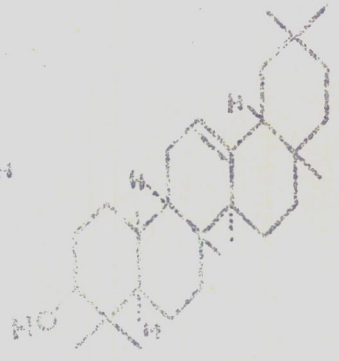
of methyl oleanolate iodo acetate has shown that (CXXVI) can be taken as representative of β amyrin.[132]



(CXXIV)



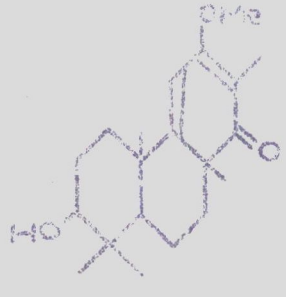
(CXXV)



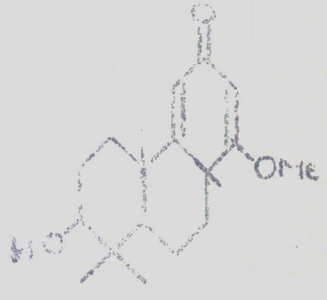
(CXXVI)

α Amyrin group:

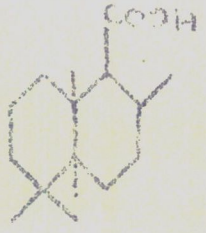
Oxidative degradation of α amyrin derivatives led to the same bicyclic ester (CVII) as obtained from oleanolic acid derivatives which indicated that rings A and B were similarly constituted in α and β amyrins.[101] Also both α and β amyrins have been converted into two isomeric methyl ethers (CXXVII) and (CXXVIII). This conversion proved the identity of the configuration at C-3, C-5, C-8 and C-10 in both the series.[133,134]



(CXXVII)

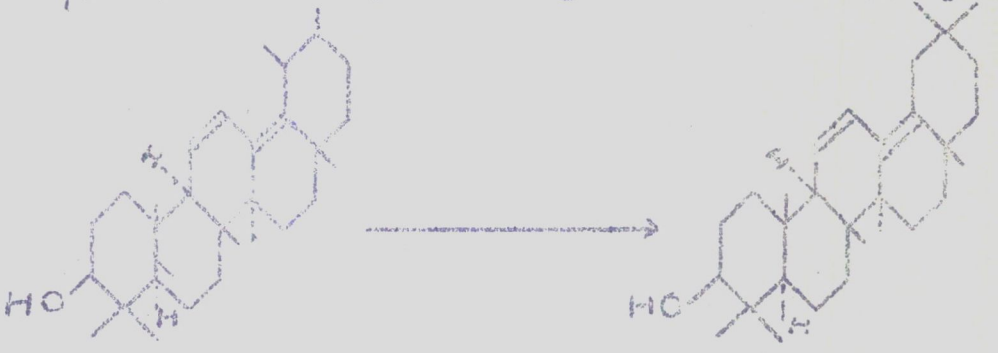


(CXXVIII)



(CVII)

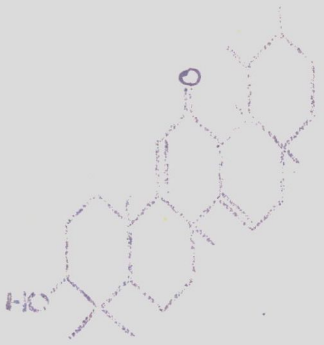
△ 11,13(18) ursadiene 3 ol (CXXIX) was converted into 11,13(18) oleadiene 3 ol (CXXX) by vigorous acid treatment of the former.[102] Since this transformation does not involve C-9, C-14 or C-17 it could be presumed that the configuration at these centers are the same as in β amyrin. Further both α and β amyrins undergo rearrangement to the corresponding



(CXXIX) (CXXX)

isodienonyl structure with selenium dioxide, but not 18-iso(18α)amyrin,[135] and hence the hydrogen at C-18 in α amyrin may be taken to be β by analogy. This formulation has been also supported by study of optical rotations and stabilities of the lactones of ursolic acid and oleanolic acid.[136]

The evidences recorded above, proves the configurations of all relevant points except those of the methyl groups at C-19 and C-20. Ruzicka and collaborators[137] isolated a hydrocarbon (CXXXII) by pyrolysis of isoamyradienonyl acetate (CXXXI) which was converted to a ketone (CXXXIII). This ketone was also obtained by degradation on D(+)-pulegone (CXXXIV) which established the structure of the ketone as well as the configuration of the methyl group at C-20 as α.[137]



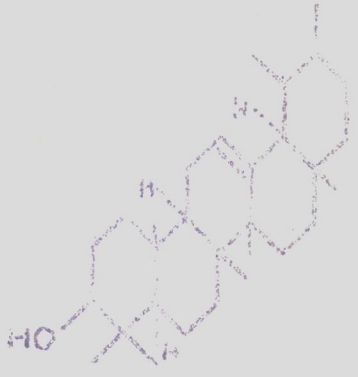
(CXXXI)

(CXXXII)

(CXXXIII)

(CXXXIV)

These evidences lead to (CXXXV) as representative of α amyrin; the methyl group at C-19 being placed in the equatorial conformation for maximum hindrance of the double bond.



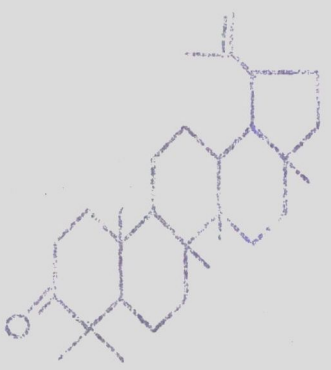
(CXXXV)

The diequatorial substituents in ring $\mathcal{A}$ may explain the relative stability of the cis D/E fusion, for, an epimerisation at C-18 should make them axial.[136]

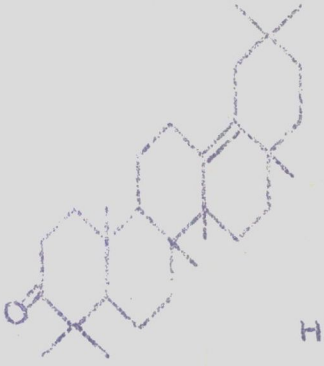
Lupeol group:

Under acidic conditions lupenone (CXXXVI) was transformed into β amyrenone (CXXXVII) which is of known constitution and has been derived from β amyrin.[138] This transformation allowed

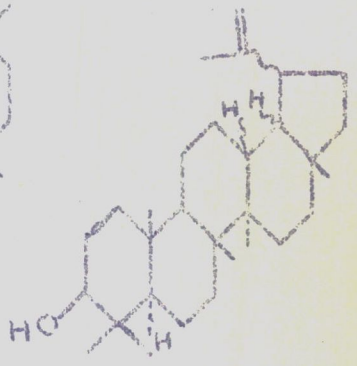
the stereochemistry of lupeol to be written as (CXXXVIII), in which the configuration at C-13, C-18 and the isopropenyl group were still to be determined.



(CXXXVI)

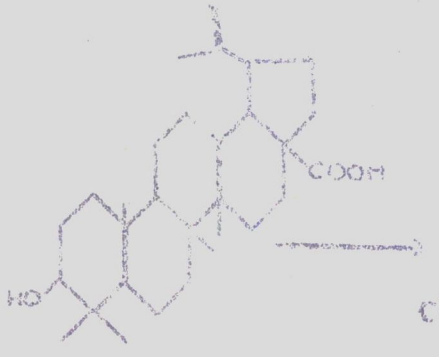


(CXXXVII)

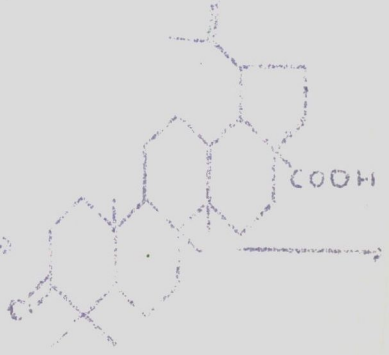


(CXXXVIII)

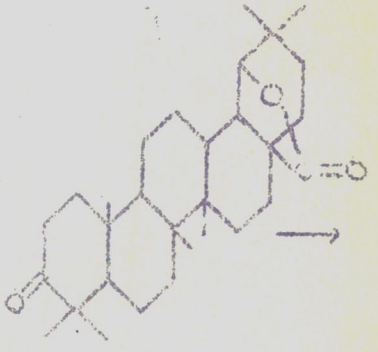
Betulinic acid (CXXXIX) is a compound which belongs to the lupeol group and which differs from lupeol in that it carries a carboxyl group at position 17. Betulonic acid (CXL) derived from betulinic acid, on treatment with acid, isomerised to a ketolactone (CXLI)[139]. Reduction of this with lithium aluminium hydride gave a triol (CXLII). The triol on acetylation with boron trifluoride and acetic anhydride furnished moradiol diacetate (CXLIII) obtained from morolic acid. Since the centre C-13 is not involved in this series of reactions the hydrogen at C-13 should have the same configuration as in morolic acid, i.e. β.[130]



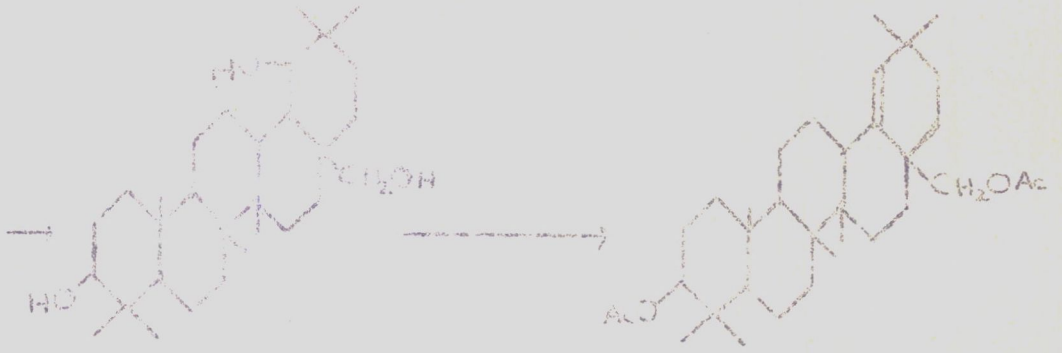
(CXXXIX)



(CXL)



(CXLI)

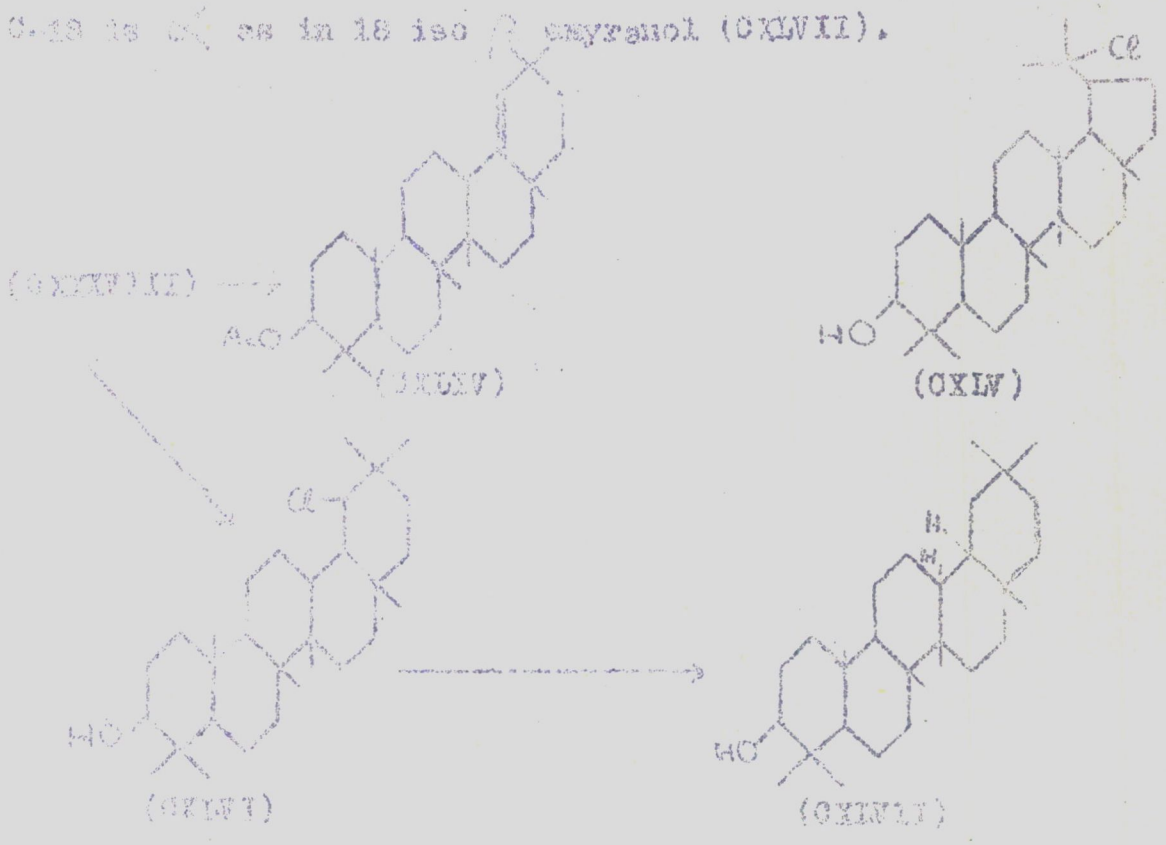


(XXXII) (XXXIII)

The case of dehydration of the 19 hydroxyl group in (XXXII) indicated that it had an axial configuration and also that the C-13 hydrogen was axial and trans to the 19 hydroxyl. Since the diol (XXXIV) was formed by the reduction of the lactone (XXXI) the 19 hydroxyl group and the C-17 primary hydroxyl should be β as we with respect to one another, and hence it followed that the 18-hydrogen was α and that the rings D and E were trans fused.

The configuration of the isopropenyl group was determined by Halsall and collaborators[130] by reactions on lupeol. Lupeol (XXXVIII) formed a hydrochloride which on treatment with silver acetate regenerated lupeol, whereas on treatment with acetic anhydride gave the acetate of germanicol (XXXIV) The hydrochloride on boiling with inert solvents was recovered unchanged but on boiling with an inert ionising solvent it gave germanicol. The hydrochloride may therefore have the structure (XXV) or (XXVI). Reduction of the hydrochloride with sodium and isopropanol or catalytically gave 18 iso β amyrenol (XXXVII) [130]. The structures (XXVI) for the

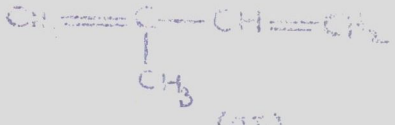
hydrochloride. This also indicated that the hydrogen at C-18 is α as in 18 iso β amyrenol (CXLVII).



A consideration of the probable mechanistic path indicates that the chloride atom in the hydrochloride is α. Thus germanicol is obtained from the hydrochloride under the conditions favouring the SN_1 mechanism and the regeneration of lupeol from the hydrochloride by the SN_2 type reaction should proceed by the path indicated (CXLVII) wherein the chloride atom, C-18, C29 and C21 are coplanar. This requires the chloride atom to be α and also for the configuration of the isopropenyl group to be trans to the methyl group at C-17

The five carbon fragment postulated has probably its origin in a two carbon fragment. Thus cholesterol has been biosynthesised from acetic acid.[141] It was later found that β hydroxy β methyl δ valero-lactone (the lactone of mevelonic acid) (CLI) is utilised in the biosynthesis of cholesterol[142] and squalene.[143] It was also found that the carboxyl group of mevalonic acid was the carbon lost in the formation of the five carbon unit.[144]

It has been postulated that each of the terpene group could be derivable from simple percursors; the percursors themselves being formed by the combination of five carbon fragments. Thus geranial (CLII) can give rise to monoterpenes farnesol (CLIII) to sesquiterpenes geranylgeraniol (CLIV) to diterpenes and squalene (CLV) to triterpenes.



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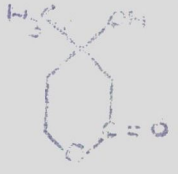
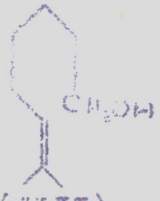
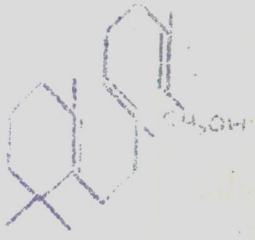


(CLI)

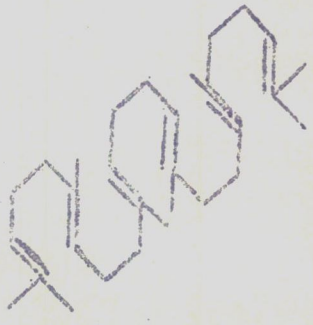


(CLII)

(CLIII)



(CLIV)

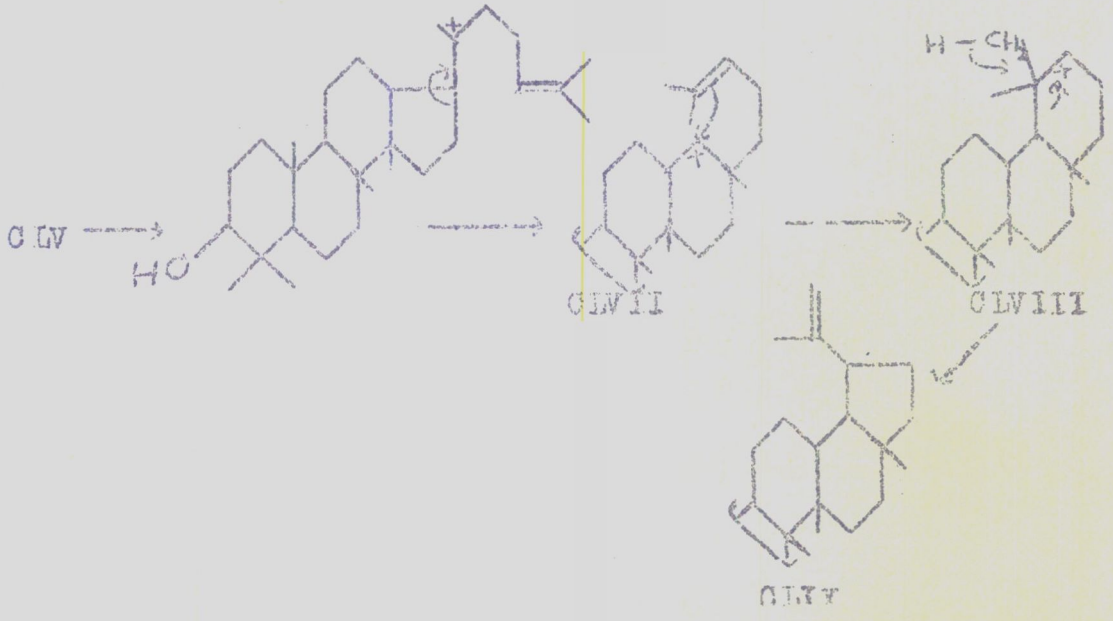


(CLV)

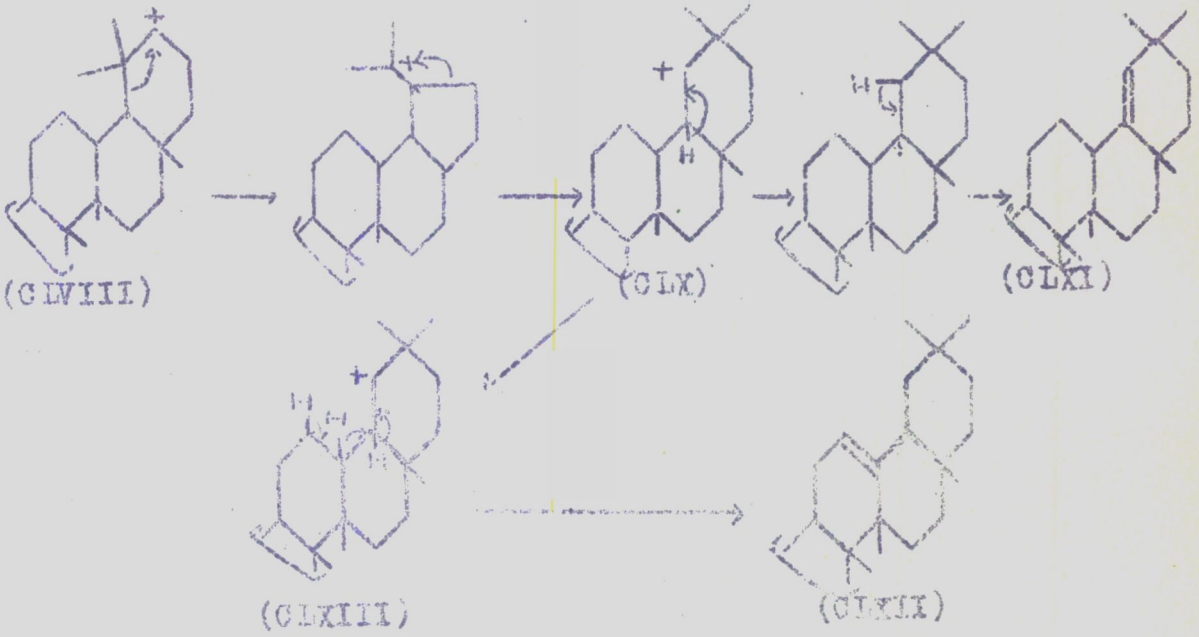
As mentioned above the triterpenes can be postulated as derived from cyclisation of squalene (CLV) and a comprehensive scheme for the biogenesis of triterpenes was put forward by Ruzicka and collaborators[145] and by Stork and Burgstahler[146], independently. Thus cyclisation of squalene (CLV) can lead to euphol (CLVI).

(CLV) (CLVI)

Squalene through the ions (CLII) and (CLVIII) gives rise to lupeol (CLIX) family.



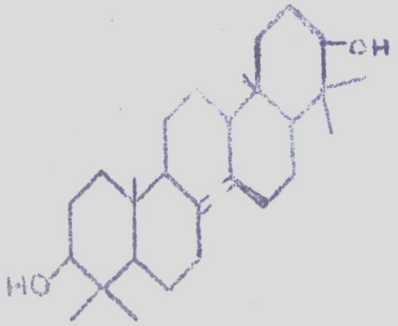
The ion (CLX) derived from the ion (CLVIII) can lead by successive shifts to germanicol (CLXI), β amyrin (CLXII) etc.



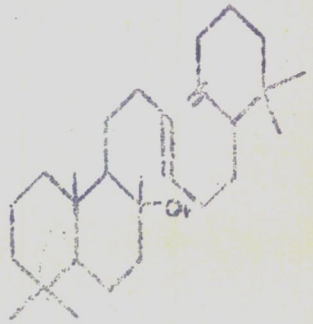
(CLVIII) (CLX) (CLXI)

(CLXIII) (CLXII)

Onocerin (CLXIII) can be pictured as a product of cyclisation of squalene from both ends and ambrein (CLXIV) by cyclisation from one end as far as the first ring only.



(CLXIII)

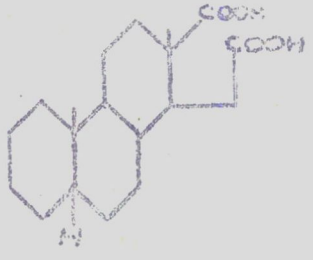


(CLXIV)

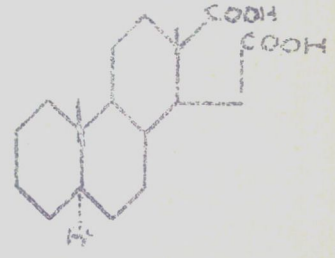
As regards the hydroxyl group incorporated in the molecule it has been found that the oxygen in the hydroxyl group is derived from the atmosphere and not from water.

Steroidal Sapogenins:

Selenium dehydrogenation of steroidal sapogenins led to Diel's hydrocarbon which showed the presence of steroidal nucleus in them[148] and these compounds were also shown to be C-27 compounds.[149,150] Further, sarsapogenin and tigogenin were degraded to etiobillianic acid (CLXV) and etiallobillianic acid (CLXVI) respectively.[149,151] The formation of etiobillianic acids while confirming the steroidal nucleus of the compounds also indicated that the side chain was attached to C-16 or C-17. The side chain was found to consist of 8 carbon atoms by the isolation of methylisohexyl ketone on the degradation of sarsapogenin.[152] This fact was further confirmed by the

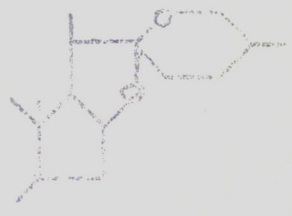


(CLXV)



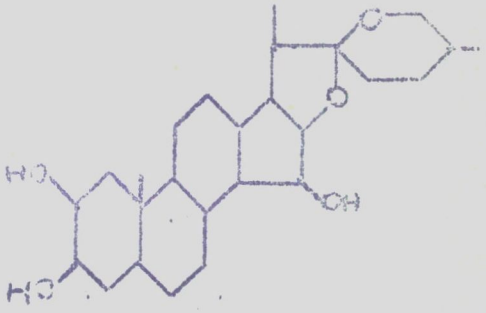
(CLXVI)

conversion of diosgenin to cholesterol.[153] The side chain which also carries two nonhydroxylic oxygen atoms was finally shown to have a spiroketal form (CLXVII) by Marker and collaborators.[154,155]



(CLXVII)

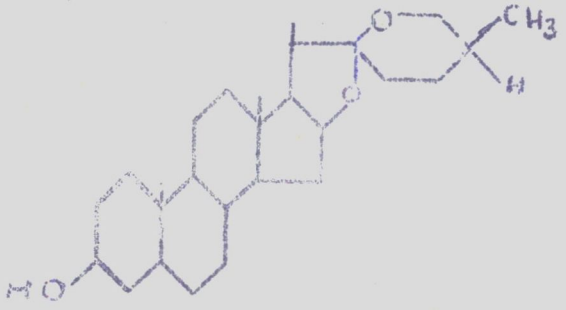
The majority of the sapogenins known are 5α compounds although an important majority are 5β. Some Δ 5 compounds are also known. In addition to position 3, hydroxyl groups are found at positions 2,6 and 12. Digitonin (CLXVIII) is unique among natural steroids in having a hydroxyl group at position 15.



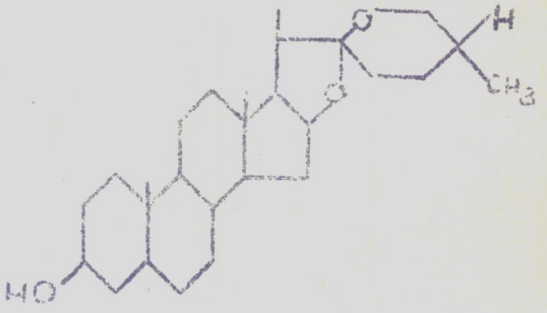
(CLXVIII)

An interesting reaction of the steroidal genins is the iso-reaction, originally discovered by Marker and Rohtmann.[155] They found that on refluxing with alcoholic hydrochloric acid sarsapogenin is converted into smilagenin. The less stable isomer like sarsapogenin in this case is designated as neo and the more stable as the iso compound. Marker regarded these isomers as C-22 epimers on the basis that both sarsapogenin (CLXIX) and smilagenin (CLXX) gave the same pseudosapogenin[156] and dihydro sapogenin. However, Scheer and collaborators[157] found that the pseudo sapogenins (CLXXI) and (CLXXII) were actually not identical and so also the dihydrosapogenins (CLXXIII) and (CLXXIV). Further the acid catalysed cyclisation of the two pseudosapogenins (CLXXI) and (CLXXII) gave back

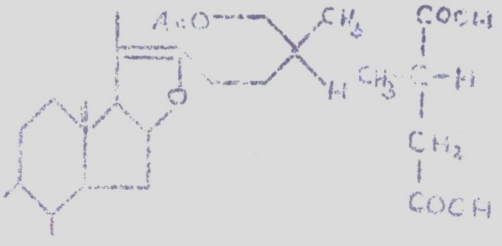
the original sapogenins and the oxidation of the pseudogenins gave different glutaric acids; 2-α-methyl glutaric acid (CLXXV) and 3-α methyl glutaric acid (CLXXVI). Finally the lithium aluminium hydride reduction of the 27 tosylates of the dihydrogenins which destroyed the assymetry at C-25 led to the same compound (CLXXVII)



(CLXIX)

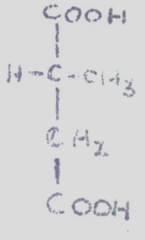


(CLXX)

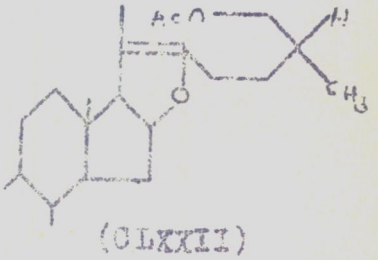


(CLXXI)

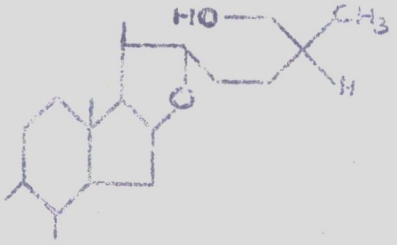
(CLXXV)



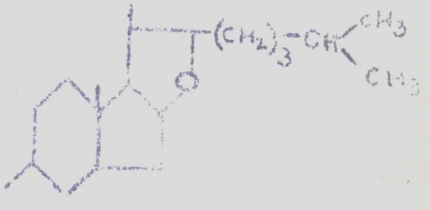
(CLXXVI)

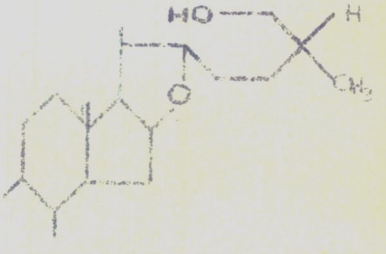


(CLXXII)

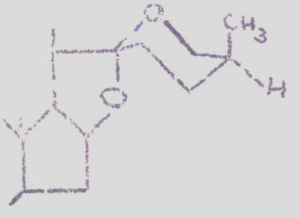


(CLXXIII)

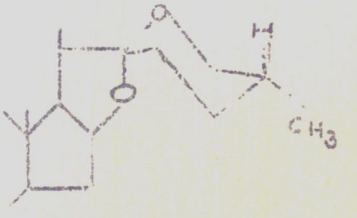




That apart from the isomerism at C-25, no isomerism is involved at C-22 was proved by Callow.[158] The controlled oxidation of neotigogenin and tigogenin acetates led to the 23 keto compounds, both of which were converted to the 24-bromides. The bromides on treatment with alcoholic potassium hydroxide yielded the same diosphenol. As no ring opening at C-22 was involved in this case it could be concluded that neotigogenin and tigogenin have the same configuration at C-22. Finally it was established that the iso-reaction was reversible.[159] Thus the neo and iso compounds are C-25 epimers and have the following conformations (CLXXVIII) and (CLXXIX).

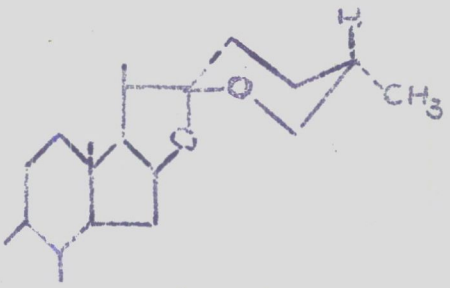


(CLXXVIII)



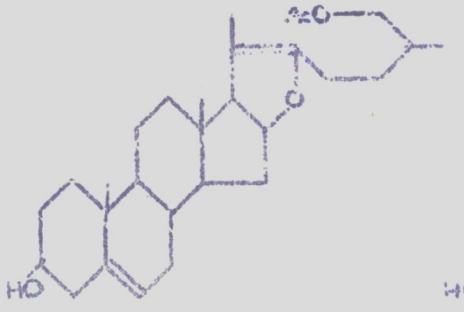
(CLXXIX)

The genins (CLXIX) give rise to pseudosapogenins (CLXXI) on treatment with acetic anhydride and the pseudosapogenins are converted back into the original genin on treatment with ethanolic hydrochloric acid.[160] However, the treatment of the pseudogenin with very mild acid or with acetic acid in ethanol gives rise to cyclopseudogenin. According to Wall the cyclo-pseudogenin has the following structure (CLXXX)[161]

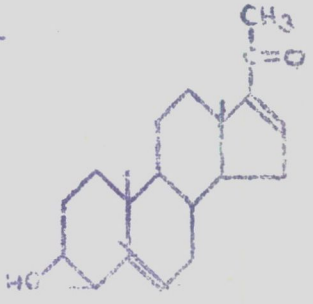


(CLXXX)

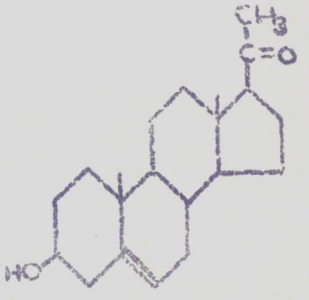
The pseudogenins are of vast importance in the chemistry of sapogenins for it is through them that the sapogenins are degraded to intermediates in the preparation of hormones.[162] Thus pseudodiosgenin diacetate (CLXXXI) on chromic acid oxidation yielded a doublyunsaturated ketone (CLXXXII) which was selectively hydrogenated to pregnenalone (CLXXXIII).



(CLXXXI)

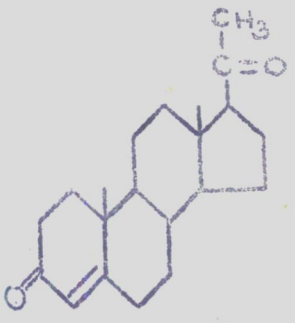


(CLXXXII)

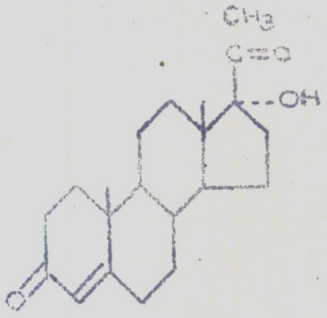


(CLXXXIII)

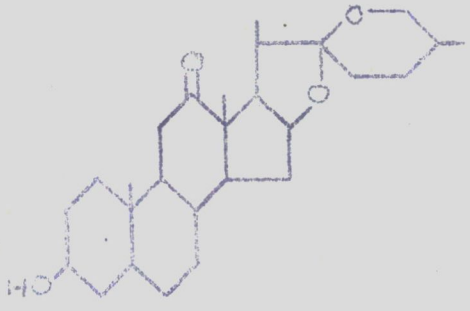
Progresterone (CLXXXIV) can be derived from pregnenolone and later it was found that progesterone can be converted to cortisone (CLXXXV).[163] Other steroidal sapogenins used in the synthesis of hormones are hecogenin (CLXXXVI)[162] and botogenin (CLXXXVII).[164]



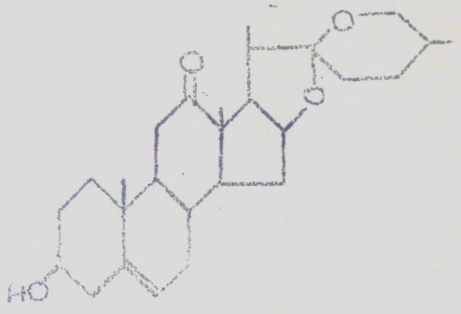
(CLXXXIV)



(CLXXXV)



(CLXXXVI)



(CLXXXVII)

Physical Methods:

The structural investigations in natural products have been greatly aided by the use of physical methods, particularly ultra-violet and infra red spectrography. Molecular rotation has found an important applications in the fields of triterpenes and steroids. The optical rotatory dispersion, N.M.R. spectroscopy and mass spectroscopy comparatively new techniques have also been put to considerable use in the determination of structures of triterpenes and steroids.

Ultra-violet spectroscopy:

The main use of ultra-violet spectroscopy is in the detection of conjugation. Emprirical rules have been proposed [165] and modified [166] for predicting the position of the maxima of absorption bands for different types of systems. Ultra-violet spectroscopy has been particularly useful in classifying triterpenes; for, members belonging to the β amyrin group on selenium dioxide oxidation yield △11,13(18) dienes showing characteristic triple maxima at 243, 251 and 260 mμ. [78] The characteristic ultra-violet maxima encountered for α,β unsaturated carbonyls and conjugated dienes in the triterpene series have been reviewed by Noller. [167]

Apart from the study of conjugated dienes and α,β unsaturated carbonyls, the spectra of steroids and triterpenes containing isolated double bonds have been examined in the range 192-250 mμ. [163] In such a study in the ultra-violet operating down to 170 mμ it was found that the △ 12 trisubstituted double bond has an absorption maxima at 194 mμ and that the tetra subtituted double bonds have characteristic absorption maxima at higher wave lengths. [169]

Infra-red spectra:

The infra red spectra of steroids have been studied in detail by many workers. [170,171] Detailed study of the infra-red spectra of the triterpenes have also been made. Thus details are available for absorption by different type of carbonyls [172]

and double bonds in pentacyclic triterpenes.[173] The infra-red absorption of equatorial and axial hydroxyls at position 3 and their acetates have also been studied.[174] Further, the infra-red frequencies and intensities of hydroxyl absorption bands in triterpenes and similar compounds have been published.[175] Studies have also been made of triterpene diols and related compounds and the presence or absence of hydrogen bonding in them.[176]

In a study of pentacyclic triterpenes it was found that the oleanene type of compounds had bands at 1392-1379, and 1370-1355 cm^{-1} and also at 1330-1315, 1306-1299 and 1267-1250 cm^{-1} whereas the ursene type had bands at 1392-1396, 1383-1370 and 1364-1359 cm^{-1} and also at 1312-1308, 1276-1270 and 1250-1245 cm^{-1}. Thus a differentiation can be made between oleanene and ursene types on the basis of infra-red spectra.[177] (Table II).

TABLE II

AU1	AU2	AU3	BU1		BU2	BU3	
							Ursolic acid
	A01	A02	B01	B02		B03	Oleanolic acid
1400		1350	1300		1250		1200

A01: 1379-1392
A02: 1355-1370
B01: 1315-1330
B02: 1299-1306
B03: 1250-1267

AU1: 1386-1392
AU2: 1370-1383
AU3: 1359-1364
BU1: 1308-1312
BU2: 1270-1276
BU3: 1245-1260

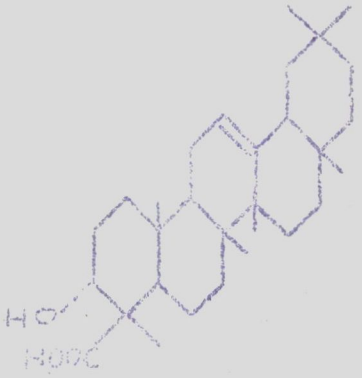
Recently attempts have also been made to differentiate between the axial and equatorial nature of carboxyl groups in triterpenes.[178]

Molecular rotation:

Apart from the utility of molecular rotation values in distinguishing between steroids and triterpenes mentioned earlier[52] these values also are applied in the structural studies of triterpenes. Thus it was found that the triterpene carboxylic acids and their methyl esters have practically the same molecular rotations.[179] It was also found that the molecular rotation values can be used to distinguish between the members belonging to the α and β amyrin groups on one hand and lupeol on the other hand.[179]

Molecular rotation values have been extended to stereochemical studies also,[180,181] and several generalisations have been made regarding the rotation contributions of hydroxyl acetoxyl and benzoyloxyl groups in alicyclic compounds.[181]

Among the numerous results obtained by the application of molecular rotations are the support for the correct stereochemistry of the β amyrin series, a correlation of the stereochemistry of the triterpenes with that of the steroids and the fixation of the configuration of the hydroxyl group at position 3 in boswellic acid (CLXXVIII).[181]



(CLXXXVIII)

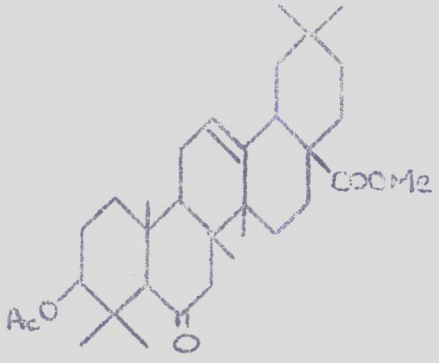
Optical rotatory dispersion:

Optical rotatory dispersion has become an important tool in the hands of the organic chemist, due to the pioneering efforts of Djerassi and collaborators. Most of the work on rotatory dispersion in organic chemistry has been conducted with carbonyl containing substances. The most important application of optical rotatory dispersion is in the study of relative and absolute configurations and in the solution of conformational problems. Rotatory dispersion is also employed in structural studies usually in the location of carbonyl group in a polycyclic system.[182]

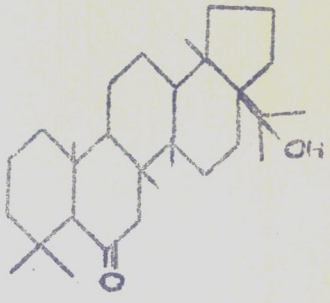
Optical rotatory dispersion curves for the steroids with keto groups in differend positions are available[182,184] and by comparison with these curves the position of the keto group in an unknown compound with a steroid nucleus can be fixed.

Similar studies have also been conducted in the triterpene field.[185] Because of the similarity of the optical rota[illegible]y

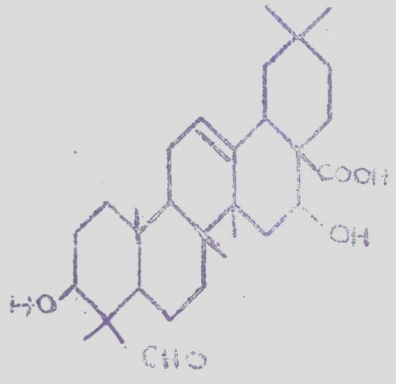
dispersion curves of methylsumaresinonate-3-acetate (CLXXXIX) and zeorinone (CXC), it has been suggested that zeorinone has also stereo-chemical arrangements corresponding with that of sumaresinolic acid.[221] Similarly quillaic acid (CXCI) and gypsogenin (CXCII) have also been concluded to be similarly constituted in ring A because of the similar rotatory dispersion curves.[186]



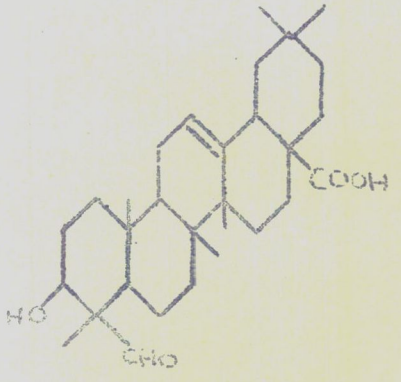
(CLXXXIX)



(CXC)



(CxCI)



(CXCII)

an acetylated triterpene absorbed between 4.0 and 4.75 ppm. while the corresponding equatorial proton absorbed between 5.0 and 5.4 ppm. Protons α to an acetylated 1,2-glycol appeared at much lower field than the protons α to isolated acetoxyl groups and gave sharper peaks. In pentacyclic triterpene Ourisson and collaborators have also studied the N.M.R. spectra of the lupane group with particular reference to the methyl absorption.[189]

Keeping such generalisations in view, it is possible to interpret a N.M.R. spectrum of a pentacyclic triterpene to advantage and arrive at definite conclusions.

Mass Spectrometry:

Application of this technique at a lower voltage (10-15 v) affords a method for accurate determination of molecular weights with only 1 mg. of the material.[190] When a higher ionising voltage (70 v) is used, fragmentation of the molecule occurs and the masses of the fragments may permit some inferences regarding the structure.

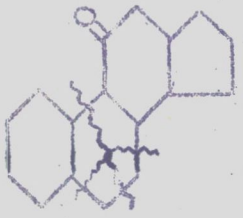
A systematic mass spectroscopic study of various natural produots such as steroids,[191] alkaloids,[192] and triterpenes[193] have been made by Djerassi and collaborators. Detailed study of various type of steroids have shown which way the fragmentation occurs.[194] Thus saturated 11-oxo steroids were found to undergo principally fission of ring B by cleavage of the 6-7 and 9-10 bonds accompanies by subsidiary peaks involving the

Nuclear magnetic resonance spectroscopy:

Pentacyclic triterpenes have many distinct absorptions in the N.M.R. spectra. Methyl esters of triterpenes give sharp absorption.[187] Angular methyl groups also give sharp absorption, but as these compounds contain a number of such methyl groups their absorption is often found to overlap.

Shamma and collaborators[188] have studied the N.M.R. spectra of a series of pentacyclic triterpene derivatives and have made some correlations between the spectra and the structures It was found that in every case in which a C-28 carbomethoxy function is present in a triterpene of the ursane or the oleanane series, the highest C methyl absorption appears upfield from 0.775 ppm. It was also found that the absorption of a C-28 methyl ester belonging to the oleanane or the ursane group is usually upfield from 3.595 ppm. while the carbomethoxyls located in other positions such as C-24 or C-30 absorb further downfield in the region from 3.595-3.650 ppm. It was also concluded that the proton of the normal trisubstituted double bond in the ursane and the oleanane series absorbs in the region between 4.93 and 5.50 ppm. Well defined and sharp vinylic methyl peaks were usually found to appear between 1.63 and 1.80 ppm. Acetoxyl protons were found to give the sharpest absorption of any function in the triterpene series; appearing between 1.82 and 2.07 ppm. A good majority of such protons were found to absorb between 1.92 and 1.97 ppm. The axial C-3 proton of

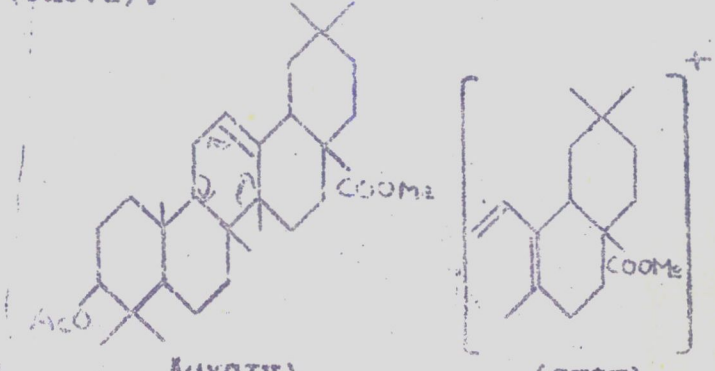
rupture of 5-6 and 9-10 linkages (CXCIII).



(CXCIII)

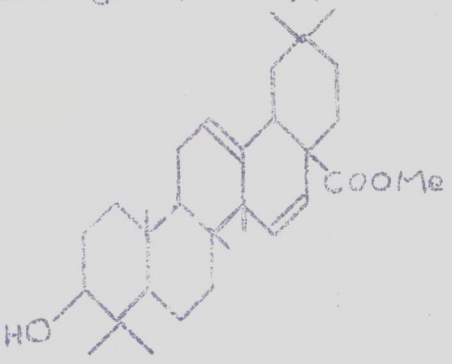
In triterpenes the principle fragmentations are associated with nucleur unsaturation. [193] While the members of the α and β amyrin class cannot be readily distinguished by mass spectrometry, lupeol can be distinguished from the former two.

The fragmentation process involved is a retro Diels Alder reaction involving the Δ 12 double bond. Thus in the mass spectra of methyl oleanolate 3-acetate (CXCIV) the most important high mass peak is that representing rings D-E and part of C (CXCV). Further loss of one of the extra nucleur substituents, in this case the carbomethoxyl function is also very characteristic giving rise to a very strong peak (CXCVI).



(CXCIV) (CXCV) (CXCVI)

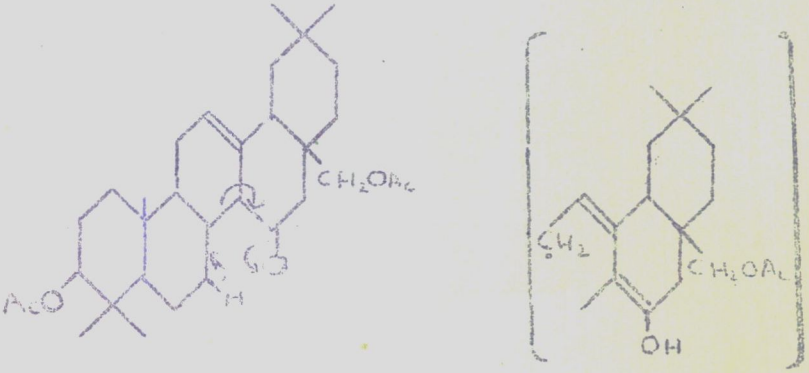
The introduction of additonal double bonds do not affect this process and the important peaks of methyl 15-dehydro oleanolate (CXCVII) was found to occur in the same region as the oleanolate analogue (CXCVI).



(CXCVII)

Occassionally the location of a particular group, notably carbonyl group can be derived from the hydrogen transfers accompanying the typical fragmentation shown above as, was found to be the case with the 15-ketones of the amyrin series (CXCVIII).

The requirement of microquantities of materials and the sensitivity in the detection of impurities makes mass spectrometry a very important tool in the hands of the organic chemist.



(CXCVIII)

The use of physical methods as mentioned above is of immense use in the chemistry of natural products, especially in the determination of stereochemistry and conformational analysis.

PRESENT WORK

PRESENT WORK

Saponins which are widely distributed in the plant kingdom are encountered in various parts of plants. India possesses a very rich flora and this work was taken up with the idea that this study on the saponins and sapogenins from various Indian plants may lead to the isolation, and characterisation of new sapogenins, determination of their structure and establishment of their biogenetic relationship, if possible. In the present work recorded in this thesis six plants have been examined, four of which belong to the same family, viz. Leguminoseae and the other two to the families Myrtaceae and Simaroubaceae.

Family:

A - Leguminoseae

Sub-family (i) Mimoseae

(1) Acacia concinna DC.
(2) Albizzia amara Benth.
(3) Pithecolobium dulce Benth.

(ii) Papilionaceae

(4) Sesbania speciosa Taub.

B - Myrtaceae

(5) Psidium guajava Linn.

C - Simaroubacea

(6) Balanites roxburghii Planch.

The members of the family Leguminoseae have been found to be a rich source of saponins and sapogenins. Recently in a paper entitled "Leguminoseae Saponins" dealing with the nature, biogenesis and other aspects, Varshney [195] has summarised the work on the various members of the family Leguminoseae (Cf: Table LXI, page 79). It has been reported [195], that in the family Leguminoseae, the sub-family Papilionaceae contain both steroidal and triterpenic sapogenins, while the members of the sub-family Mimoseae, from which the maximum number of plants have been studied, contain only triterpenic sapogenins. It has also been noted that sapogenins are absent in the sub-family Caesalpinieae.

Varshney [195] has also found that in all the triterpenes isolated from this family, oxygenation takes place chiefly in rings D and E at positions 16 and 21 in addition to ring A and that almost all the triterpenes isolated from this family belong to the β-amyrin group. It has also been concluded on the basis of the above study that oxygenation is a result of subsequent and secondary process and that it is not connected with the formation of the carbon skeleton.

It has also been found that the sapogenins from the same plant collected from different localities can differ in its yield and also in chemical nature, and therefore it has been suggested that in the botanical identification of plants the nature of the chemical constituents should also be considered. It was also found that various parts of the same plant can contain the same or different genins.

The following pages describe the work on six plants detailed above.

TABLE III

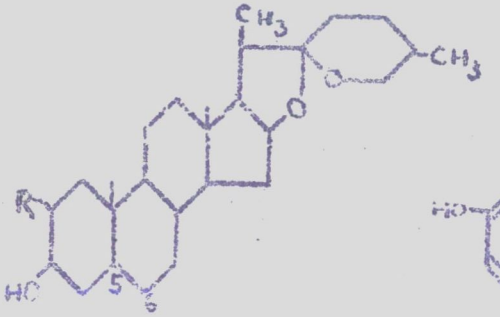
S.No.	Name of the Plant	Origin	Part Studied	Sapogenins identified	References
1.	Albizzia lebbek Benth	U.P. (India)	Seeds	Oleanolic acid (CXCIX) Echinocystic acid (CC)	196,197
2.	Albizzia lebbek Benth	M.P. (India)	Seeds	Oleanolic acid (CXCIX) Echinocystic acid (CC)	198
3.	Albizzia lebbek Benth	U.P. (India)	Beans w/o seeds	Oleanolic acid (CXCIX) Echinocystic acid (CC)	198
4.	Albizzia lebbek Benth	Bengal (India)	Complete beans	Oleanolic acid (CXCIX) Echinocystic acid (CC) Albigenic acid (CCI) Albigenin (CCII)	199
5.	Albizzia lebbek Benth	U.P. (India)	Bark	Acacic acid (CCIII)	200
6.	Albizzia lebbek Benth	U.P. (India)	Flowers	Echinocystic acid (CC) Quercetin (CCIV)	201

7.	Albizzia procera Benth	Maha-rashtra (India)	Seeds	Proceric acid (CCVI)	203
8.	Albizzia odora-tissima Benth	U.P. (India)	Seeds	Echinocystic acid (CC)	204
9.	Albizzia odora-tissima Benth	Maharash-tra (India)	Seeds	Machaerinic acid (CCV)	205
10.	Albizzia procera Benth	M.P. (India)	Seeds	Machaerinic acid (CCV)	202
11.	Albizzia anthel-mentica	Germany	Bark	Echinocystic acid (CC)	206
12.	Albizzia amara Benth	Kerala (India)	Seeds	Echinocystic acid (CC)	207
13.	Albizzia lucida	Bengal (India)	Seeds	Echinocystic acid (CC)	208
14.	Albizzia stipu-lata Boiv.	Maharash-tra(India)	Seeds	Acacic acid (CCIII)	209

...

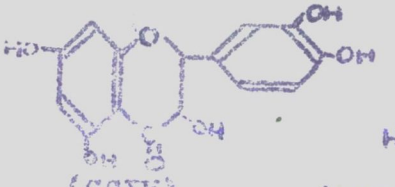
15.	Albizzia moluccana	Kerala (India)	Seeds	A new triterpenic acid A new triterpenic neutral genin	210
16.	Acacia intsia Willd.	Kerala (India)	Bark	Acacic acid (CCIII) Acaciol (?) Lupeol (CCVII)	211-13
17.	Acacia intsia Willd.	Kerala (India)	Seeds	Acacic acid (CCIII)	212
18.	Acacia concinna DC.	Kerala (India)	Seeds	Acacic acid (CCIII)	212
19.	Acacia concinna DC.	Kerala (India)	pods	Acacic acid (CCIII)	207
20.	Sesbania aegyptica Pers.	Mahrashtra, U.P. (India)	Seeds	Oleanolic acid (CXCIX) A neutral genin	214
21.	Sesbania aculaeta Pers.	U.P. (India)	Seeds	Oleanolic acid (CXCIX) A neutral genin	215
22.	Sesbania speciosa Taub.	Kerala (India)	Seeds	Oleanolic acid (CXCIX)	207

23.	Pithecolobium dulce Benth.	Kerala (India)	Seeds	Procoric acid (CCIV) An acid genin	207
24.	Trigonella Foenum-graecum	U.P. (India)	Seeds	Diosgenin (CCVIII) Gitogenin (CCIX) Quercetin (CCIV) 3 other flavonoids	210
25.	Trigonella corniculata	U.P. (India)	Seeds	A steroidal genin two flavonoids	210
26.	Styphnodendron coriaceum Benth.	Amazon	Seeds	Styphnodendron sapogenin B (CCX) Styphnodendron sapogenin F (CCXI)	216

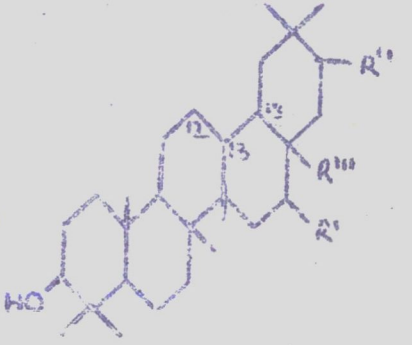


(CCVIII) R=H

(CCIX) R=OH



(CCIV)



(CXCIX) $R'=R''=H$ Δ^{12}; $R'''=COOH$

(CC) $R'=OH(\alpha)$; $R''=H$; Δ^{12}; $R'''=CH_2OH$

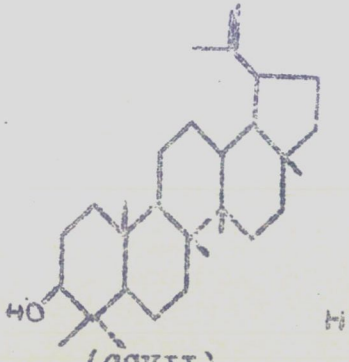
(CCI) $R'=OH$; $R''=H$ $\Delta^{13,18}$; $R'''=COOH$

(CCII) $R'=O$; $R''=H$ $\Delta^{13,18}$; $R'''=H$

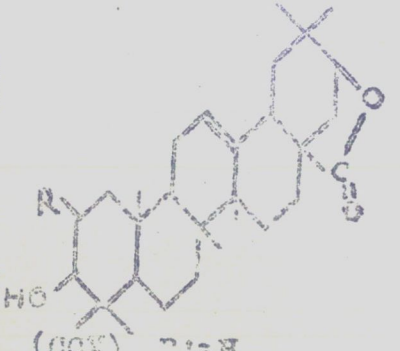
(CCIII) $R'=R''=OH$; Δ^{12}; $R'''=COOH$

(CCV) $R'=H$; $R''=OH$; Δ^{12}; $R'''=COOH$

(CCVI) $R'=H$; $R''=OH$ Δ^{12}; $R'''=COOH$
(Ring B in boat form
D in quasiboat form
and D-E ring fusion
different)



(CCVII)



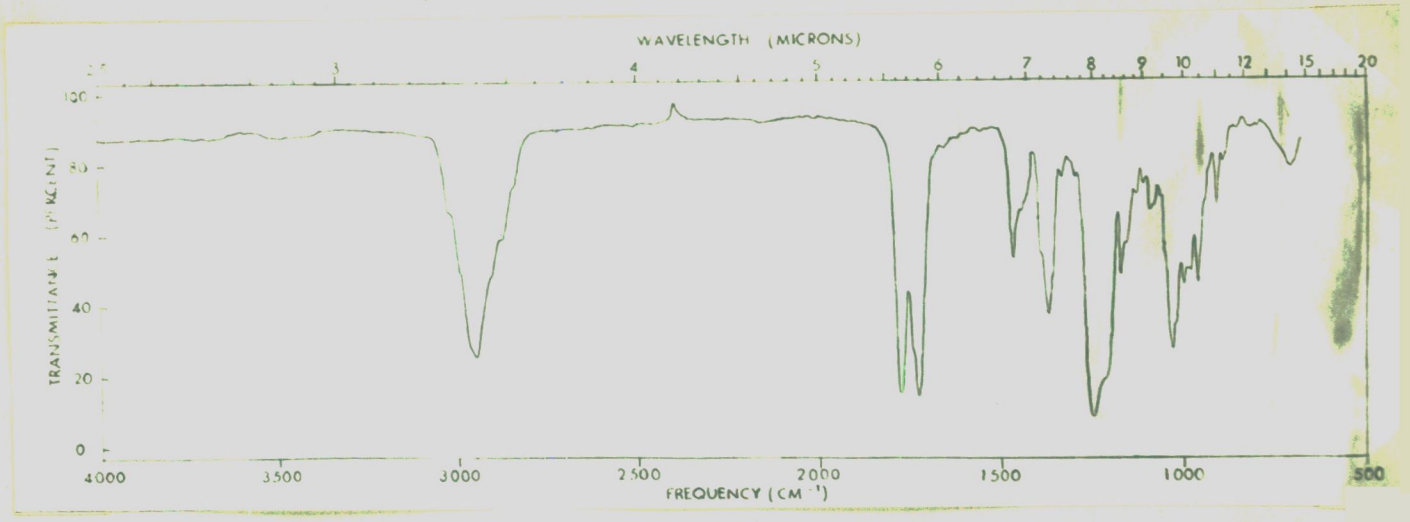
(CCX) R'=H

(CCXI) R'=OH

1. Acacia concinna DC:

Acacia concinna DC commonly known as 'Shikakai' in Hindi is a member of the family Leguminoseae, sub-family Mimoseae. It is a common prickly scanden bush. It occurs in the tropical jungles and especially in the Deccan. The pods of this plant contain 6-10 seeds and when dry appear brown and wrinkled. The pods are extensively used as a detergent and is preferred to soap when having an oil bath and by ladies for washing hair. The pods are said to be used in North Bengal for poisoning fish. The tender leaves are acid and is made use of in chutneys. The bark is used for tanning fishing lines.[217]

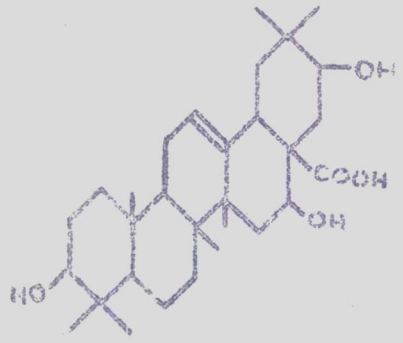
The seeds of Acacia concinna has been studied earlier[212] in these laboratories and found to contain a triterpenic acid, Acacic acid m.p. 275-76°; acetate m.p. 235-36°. Acacic acid was earlier isolated from the bark of Acacia [211,218] catechu and was thought to be a trihydroxy mono carboxylic acid, belonging either to the α-amyrin or tetracyclic triterpene group,[211,218] but later Varshney[195,219] on biogenetic grounds suggested the 3,16,21-trihydroxy olean-12-ene 28-oic acid (CCXII) structure for this acid.



(Fig. I)

Genin:

The acetate m.p. 235-36° was deacetylated by refluxing with alcoholic potassium hydroxide (10%), and the isolated product crystallised from a large volume of methanol as colourless needles, m.p. 278-82°. It gave a positive reaction with tetranitromethane. The melting point of the acid genin and its acetate were in quite agreement with the melting points of acacic acid and its acetate (Cf: acacic acid m.p. 280-81°; acetate m.p. 234-35°)[211] and



(CCXII)

It has been established by Varshney[195] that the saponins and sapogenins from various parts of the same plant or the same plant from different localities can vary not only in their yield but also in the chemical nature. No work has been done on the pods of this plant apart from attempts on the biochemical standardisation of the saponin content[220] and the standardisation of the conditions for the isolation of the crude saponin.[221] Therefore this work was taken up, as nothing was known about the chemical nature of the saponin and sapogenin from the pods. During the course of this work another communication appeared, again on the standardisation of the conditions for the isolation of the crude saponin, but this also did not report anything about the structure of the saponin or the sapogenin.[222]

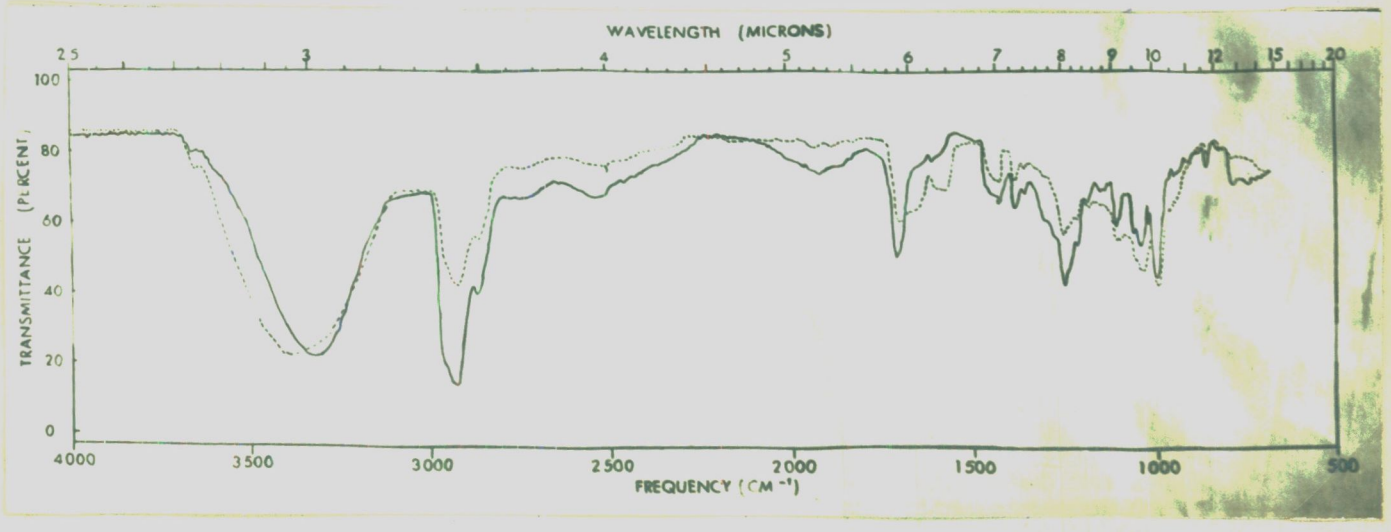
The well powdered pods of Acacia concinna, obtained from Kerala were exhausted with alcohol. The recovery of the solvent yielded a dark brown coloured semi-solid residue. This was successively treated under reflux with petroleum ether, ether, chloroform, carbontetrachloride and acetone to remove the impurities soluble in these solvents. The residue thus obtained was dissolved in the minimum quantity of alcohol and the saponin precipitated by addition to a large volume of ether or acetone. This process of dissolution and precipitation was repeated a number of times till a fairly pure sample of the saponin was obtained. The saponin was then dissolved in a large amount of water and hydrolysed with sulphuric acid (10%), by heating on a water-bath for one hour followed by refluxing for another hour. The solid sapogenin formed was filtered, washed free of acid and dried. All attempts to crystallise it failed and therefore it was subjected to further purification. Thus the sapogenin was refluxed with alcoholic potassium hydroxide solution (10%) for one hour, the volume concentrated to half and the solution diluted with a large amount of water, which was then extracted with ether. The ether extracts were washed free of alkali and recovered to yield a neutral portion in traces only. The alkaline aqueous layer was acidified

with hydrochloric acid which precipitated the acid genin. It was filtered, washed free of acid and dried.

Acetate:

As all attempts to crystallise this acid genin failed, it was acetylated by two different methods, i.e. by acetic anhydride and pyridine in the cold and acetic anhydride and sodium acetate, with identical results. The products worked out in the usual manner on repeated crystallisations from methanol gave fine colourless needles, m.p. 235-36°. It showed a positive tetranitromethane test for carbon-carbon double bond. The infrared spectra of the acetate (Fig. 1) in chloroform showed bands of acetate and γ-lactone bands at 8.05 μ and at 5.6 μ. It also showed the presence of a triply substituted double bond between 11.8 and 12.4 μ. The mass spectra of the compound showed its molecular weight to be 454 corresponding to the formula, $C_{34}H_{50}O_6$. The general fragmentation behaviour was in league with the fragmentation pattern associated with triterpenes.

therefore mixed melting points with authentic samples of acacic acid and its acetate were taken which showed no depression. Further the infrared spectra of the genin and the authentic sample of acacic acid were identical (Fig. II).[211]



..... Present genin

_____ Authentic sample of Acacic Acid

(Fig. II)

Methyl ester:

Acacic acid on methylation with diazomethane gave a methyl ester as colourless needles, m.p. 224-25°. It gave a yellow colour with tetranitromethane and showed no depression on melting with an authentic sample of acacic acid methyl ester. (Cf. Acacic acid methyl ester m.p. 223-24°).[211] The

infrared spectra of the methyl ester (Fig. IV) showed the presence of free hydroxyl and methyl ester groups.

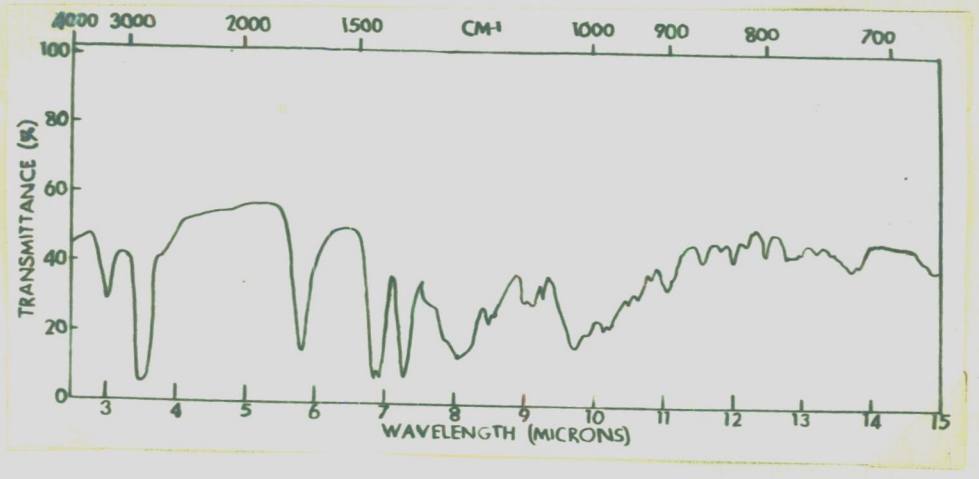
Acetyl methyl ester:

The methyl ester was acetylated with acetic anhydride and pyridine in cold and the product worked out in the usual manner. On crystallisation from methanol it gave colourless needles m.p. 203-5°. (Cf. acacic acid acetyl methyl ester[211] m.p. 208-9°). It did not depress the melting point of an authentic specimen of acacic acid acetyl methyl ester.[211] It also gave a positive test with tetranitromethane. A comparison of the physical constants of acacic acid and its derivatives with the present genin is shown below (Table IV).

TABLE IV

	Present genin	Acacic acid
1. Genin	278-82°	280-81°
2. Acetate	235-36°	234-35°
3. Methyl ester	224-25°	223-24°
4. Acetyl methyl ester	203-5°	208-9°

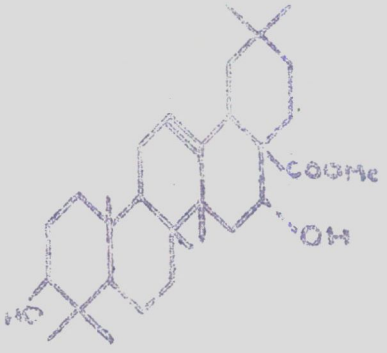
The infrared spectra of the acetyl methyl ester (Fig. III) showed a hydroxyl band in the 3 μ region, thereby indicating that atleast one of the hydroxyl groups was not acetylated.



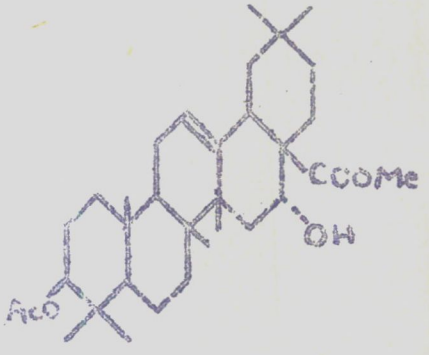
(Fig. III)

The above postulation was confirmed by the elemental analysis of the compound, wherein it corresponded to the formula of a diacetyl monohydroxy methyl ester, $C_{35}H_{54}O_7$ This behaviour of the methyl ester in forming a diacetyl methyl ester on acetylation and not a triacetyl methyl ester is paralleled by that of methyl echinocystate (CCXIII) which forms only a mono-acetyl methyl ester (CCXIV) and not

a diacetyl methyl ester. [196,197]



(CCXIII)



(CCXIV)

All these evidences showed that acacic acid is a trihydroxy monocarboxylic acid, with the formula $C_{30}H_{48}O_5$.

Relation to β-amyrin group:

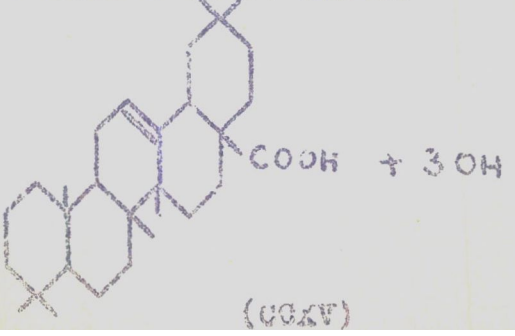
The relation of acacic acid to β-amyrin was fixed by the easy formation of a bromlactone m.p. 259-62° by the action of bromine on acacic acid in acetic acid. The product did not give any colour with tetranitromethane. The formation of a bromolactone is indicative of the oleanene type skeleton [73] and fixed the position of the carboxyl group at C-28 and the double bond at C-12-13.

The infrared spectra in pyridine has been utilised to distinguish between the acids of β and α-amyrin groups

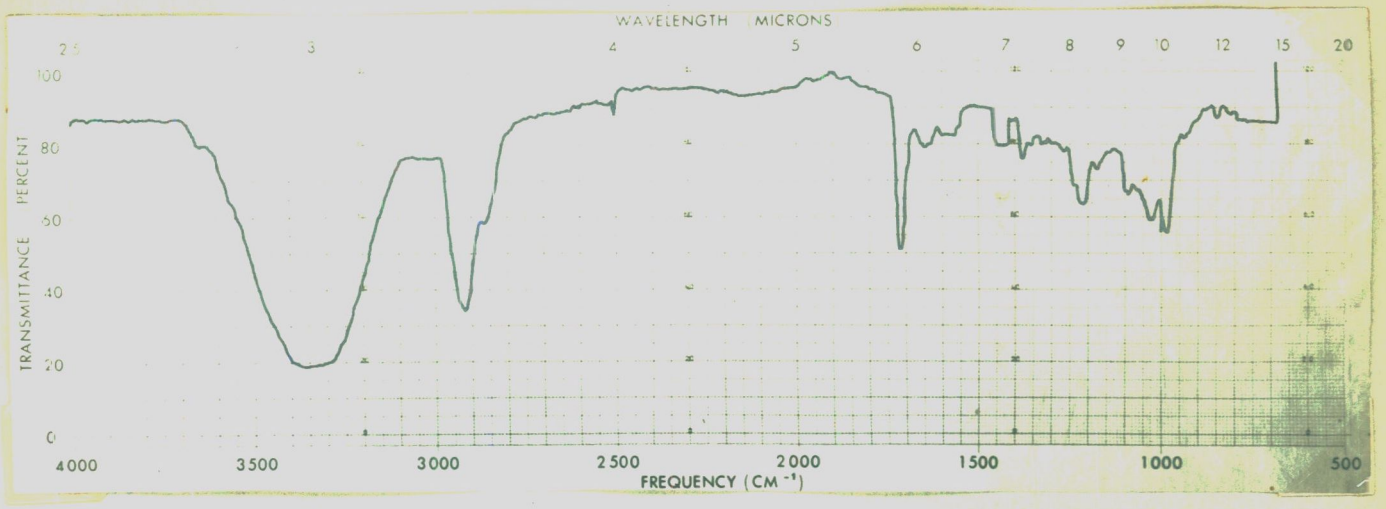
(i.e. oleanolic and ursolic acid derivatives), on the basis of their absorption in two different regions; region 'A' corresponding to 1392-1350 cm^{-1} and region 'B' corresponding to 1330-1345 cm^{-1}. The members of the oleanolic acid group have two bands in the 'A' region (1392-1379, and 1370-1365 cm^{-1}) and three bands in the 'B' region (1330-1315, 1306-1299 and 1267-1250 cm^{-1}), while the members of the ursolic acid group possesses three bands both in the 'A' (1399-1386, 1383-1370 and 1364-1359 cm^{-1}) and 'B' (1312-1308, 1276-1270 and 1250-1245 cm^{-1}) regions. In the case of acacic acid methyl ester (Fig. IV) the infrared spectra taken under the same conditions as described,[177] through the courtesy of Prof. Tschesche himself showed only two absorptions at 1380 and 1355 cm^{-1} in region 'A' and three absorptions at 1330, 1300 and 1267 cm^{-1} in the 'B' region clearly indicating its relation to the β amyrin group.

On the basis of the above evidences acacic acid can be attached to β amyrin group (oleanolic acid group) with a carboxyl group at position 28 and a double bond at C 12-13, with three hydroxyl groups (CCXV).

With the skeleton and the position of carboxyl group tentatively fixed as shown, the positions of the three hydroxyl groups remained to be fixed.



(CCXV)



(Fig IV)

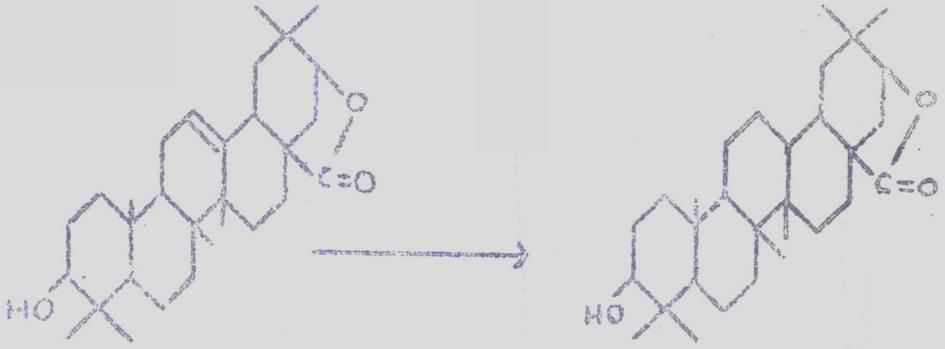
The relative positions of the functional groups:

In order to fix the relative positions of the functional groups, the methyl ester was subjected to periodate oxidation, but the methyl ester was recovered unchanged indicating the absence of a 1,2-diol system in the compound. Similarly the methyl ester failed to form an isopropylidene derivative, which while confirming the above observation also indicated the absence of a 1,3-diol system. (Cf. tomentosic acid (LVI).

The formation of a diacetyl γ-lactone as shown by the γ-lactone band in I.R. spectra (Fig. I) by acetylation of acacic acid, suggested that one of the hydroxyl groups should be situated at a position γ to the carboxyl group.

The formation of a diacetyl methyl ester, instead of the expected triacetyl derivative on acetylation of the methyl ester, parallaling the formation of a monoacetyl derivative of methyl echinocystate (CCXIV), suggested the possible location of the second hydroxyl group at a position β to the carboxyl group. The failure of the methyl ester to undergo periodate oxidation and to form an isopropylidene derivative also suggested that these two hydroxyl groups are not in a 1,2-diol system. The third hydroxyl was taken to be the ubiquitous one, at position 3.

An interesting reaction of aceanic acid acetyl lactone is that it easily undergoes hydrogenation at atmospheric pressure in the presence of Adam's platinum oxide catalyst in acetic acid solution, contrary to the normal behaviour of the members belonging to the βamyrin group. The other instance of easy hydrogenation of a member of the βamyrin group is the sapogenin 3 of Styphnodendron coriaceum (CCXVI).[216] This reaction has been explained on the basis of certain conformational features. This similarity in the behaviour of these compounds towards catalytic hydrogenation while corroborating the findings of infrared spectroscopy, also suggested the possibility of the location of the



(CCXVI)

hydroxyl group γ to the carboxyl group at position 21.

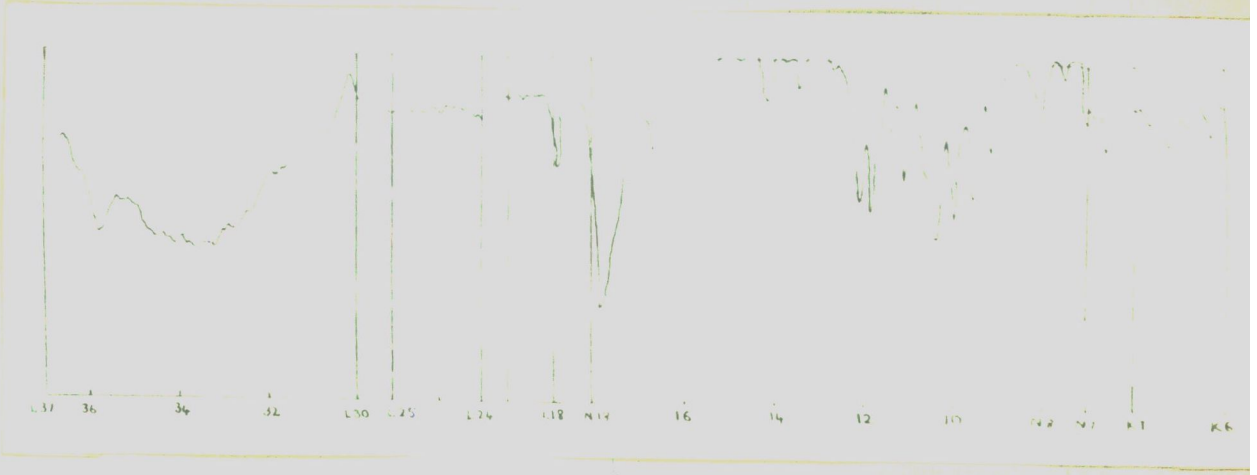
Once the relative positions of the functional groups were known, degradative studies were carried out to fix the exact location of these groups.

Position of the functional groups:

In order to preferentially manipulate the functional groups, an attempt was made to subject the diacetyl-lactone to partial hydrolysis with potassium carbonate in methanol, but the product isolated m.p. 307-20° could not be characterised properly, in spite of chromatography on acid alumina.

As the mild alkaline hydrolysis of the lactone was unsuccessful it was subjected to acid hydrolysis. Thus the diacetyl lactone was refluxed with methanolic hydrochloric

acid (10%) for two hours, the product worked out and crystallised from methanol as long needles m.p. 252-54°. It gave yellow colour with tetranitromethane. The infrared spectra of this compound (FigIVa) showed bands corresponding to hydroxyl group at 3400 cm^{-1} and γ-lactone at 1785 cm^{-1}.



(Fig. IVa)

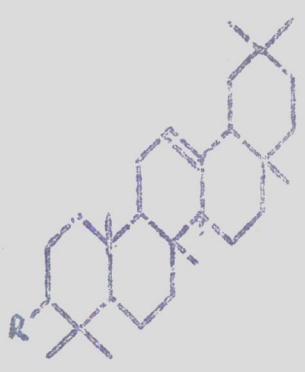
The NMR spectra of the compound (Fig. V) showed signals corresponding to one vinyl proton at 5.38 ppm. as a triplet characteristic of the protons of the β-amyrin group. One proton appearing at 4.2 ppm. as a doublet should be a lactonic proton (CH-O-CO). Two hydroxylic protons appeared at 1.53 ppm as singlets. This proof suggested that the acid hydrolysis product of the diacetyl lactone (CCXVII) can be formulated as a dihydroxy lactone (CCXVIII) as shown below.

(Fig. V)

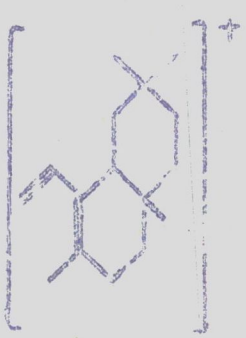
(XXXVII)

(XXXVIII)

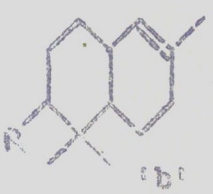
In the mass spectra (Fig. VI) of this compound (CCXVIII) the M^+ peak corresponds to the molecular weight 470 which is in definite agreement with the structure (CCXVIII), with the formula $C_{30}H_{46}O_4$. There is a peak at M-18 at M/e 452 arising out of the loss of one molecule of water. The normal fragmentation pattern associated with triterpenes are as shown below. Δ - 12 unsaturated olefines (CCXIX) undergo predominantly retro Diels Alder reaction leading to fragments 'a' and 'b'. Among other type of fragmentations, rings A and B lead to the fragment 'g'.



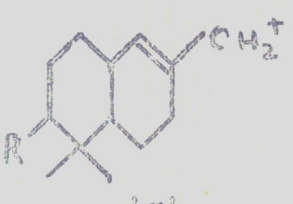
(CCXIX)



'a'



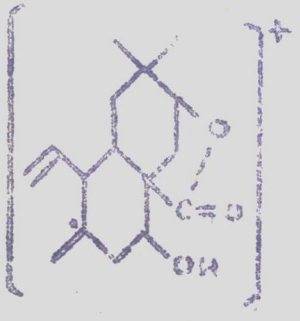
'b'



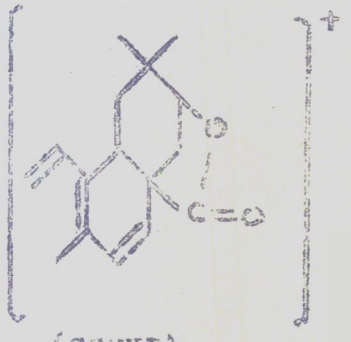
'g'

The mass spectrum of the present compound when interpreted in the light of the above observations is in exact agreement with the structure proposed. Thus there is a peak at m/e 263, which should be due to the fragment(CCXX) and a peak at m/e 244 due to the fragment (CCXXI) which

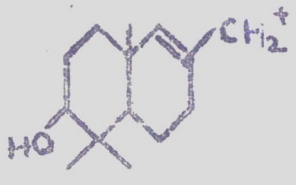
corresponds to the fragment 'a' described above. Another peak at m/e 207 due to the fragment (CCXXII) corresponds to the fragment 'g'. Other fragments arising out of rings A and B are at m/e 190 (CCXXIII) which corresponds to the fragment 'b' and a peak at m/e 175 which can be attributed to the m/e 190 fragment minus a methyl group.



(CCXX)



(CCXXI)



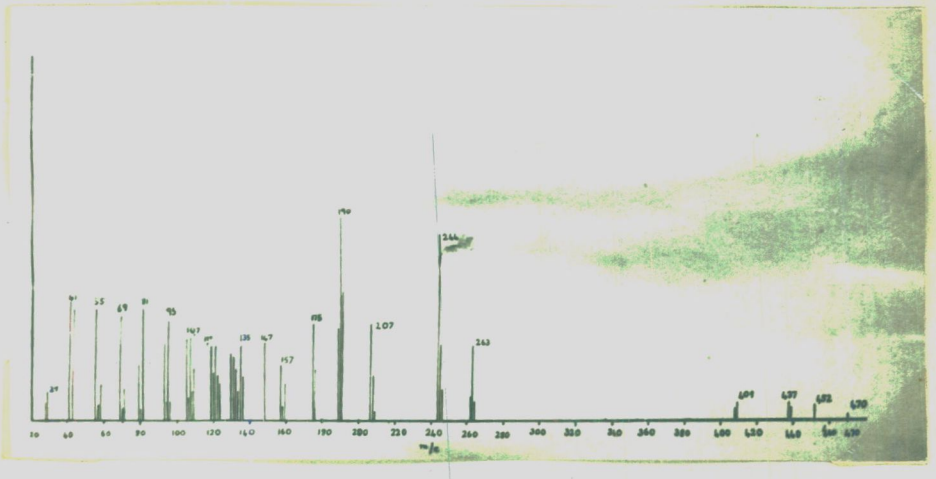
(CCXXII)

(CCXXIII)

Thus the mass spectra is in complete agreement with the structure (CCXVIII) and corroborates it.

The formation of a dihydroxy lactone (CCXVIII) on acid hydrolysis of the diacetyl lactone (CCXVII) was further confirmed by the observation, that acetylation of the dihy[illegible]xy lactone (CCXVIII) led back to the diacetyl lactone(CCXVII).

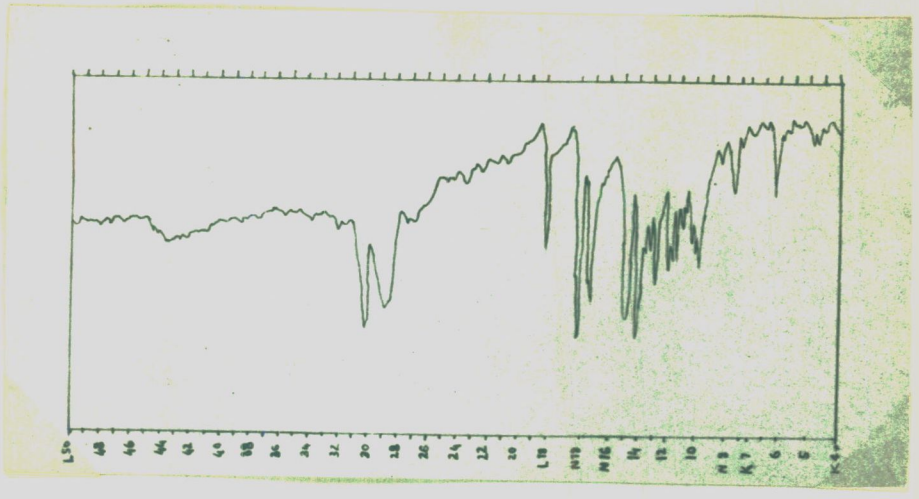
as indicated by mixed melting point.



(Fig. VI)

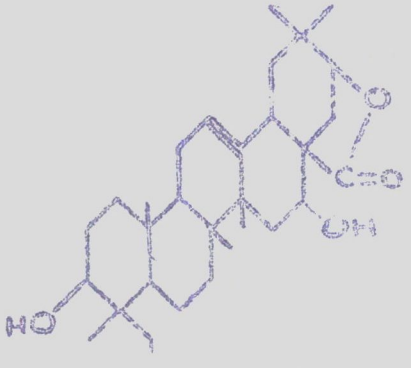
The compound (CCXVIII) was oxidised with pyridine-chromiumtrioxide complex, the product worked out and crystallised from methanol:chloroform as plates, m.p. 338-42°. It gave a yellow colour with tetranitromethane and formed a semicarbazone, m.p. 278-82°.

The infrared spectra of this compound (Fig. VII) showed no absorption in the hydroxyl region. In the carbonyl region it showed two peaks at 1720 cm^{-1} [illegible] 1310 cm^{-1}, which could be attributed to a γ-lactone and a six membered ketone respectively. Further it showed the presence of a trisubstituted double bond between 847 and 806 cm^{-1}.

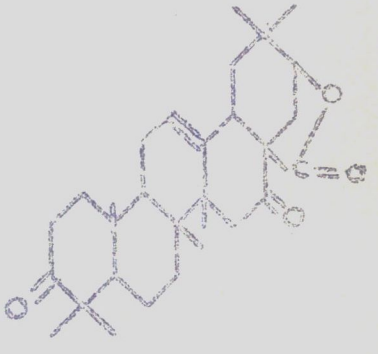


(Fig. VII)

On the basis of the structure of the dihydroxy lactone (CCXVIII) this compound can be formulated as (CCXXIV).



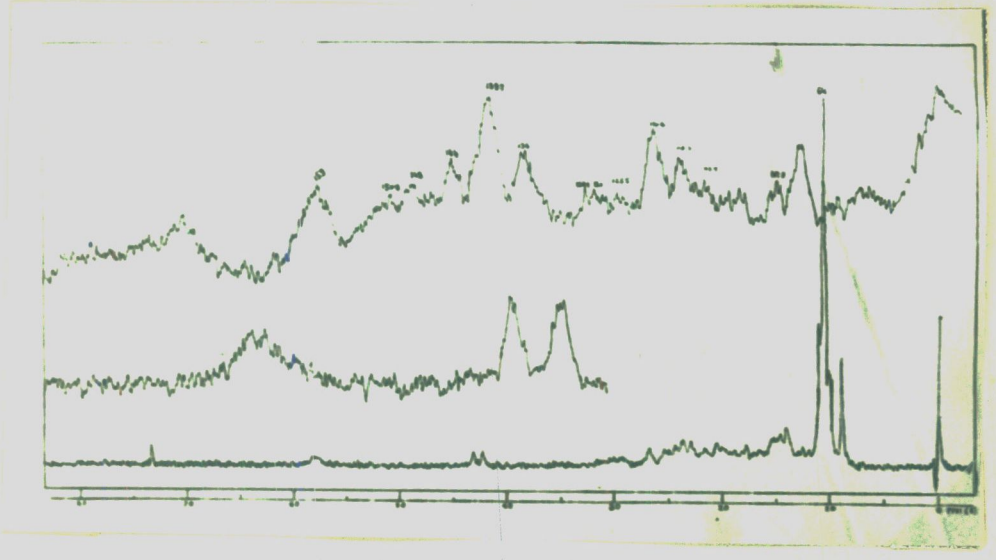
(CCXVIII)



(CCXXIV)

The NMR spectra of this compound (Fig. VIII) shows agreement with the above structure (CCXXIV). The signals

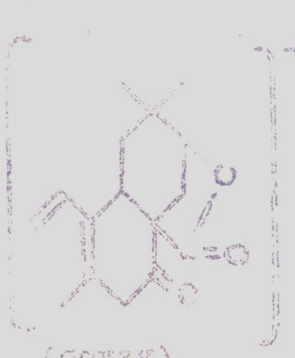
of a proton at 4.2 ppm as doublet is due to the lactonic proton as in the case of (CCXVIII). The spectra also shows the presence of the vinylic proton at 5.3 ppm.



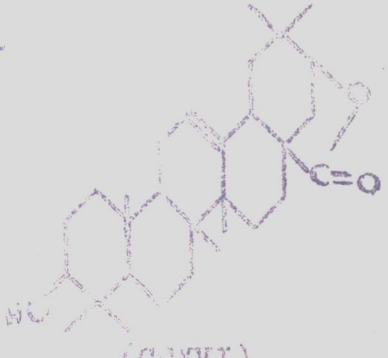
(Fig. VIII)

The mass spectra (Fig. IX) of this compound also confirms this postulation. It has the M^+ peak at 456 which is in entire agreement with the formula $C_{30}H_{48}O_4$, for the diketolactone. The peak at m/e 441 should correspond to M-15, due to the loss of a methyl group and the peak at m/e 438 to M-30, due to the loss of two methyl groups. The peak at m/e 260 is due to the normal fragmentation procedure and corresponds to the 'a' type fragment represented in this case by (CCXIV). As in the case of the sapogenin B of Styphnodendron coriaceum (CCXVI) which has a lactone

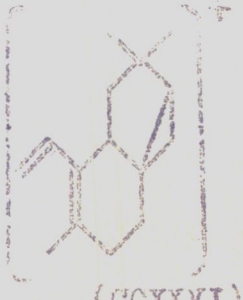
function between the carboxyl at C-28 and the hydroxyl at C-21 and which leads to the fragment (CCXXVI), this compound also gives rise to the fragment (CCXXVII) corresponding to m/e 215.



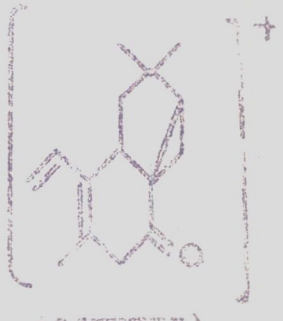
(CCXXV)



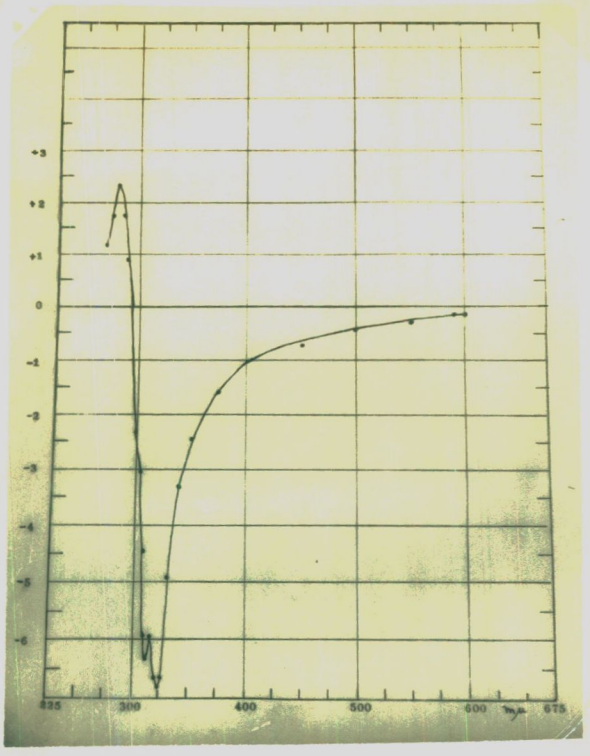
(CCXXVI)



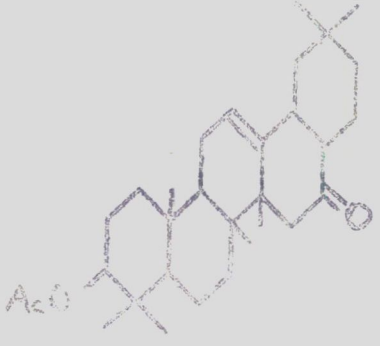
(CCXXVII)



There is a peak at m/e 180, which has been assumed to be due to a small quantity of the 11-keto product (CCXXVIII) formed by allylic oxidation of the dihydroxy lactone (CCXVIII) and whose molecular formula is $C_{30}H_{40}O_5$. The presence of traces of the 11-keto product (CCXXVIX) was also detected on the thin layer chromatography of the product (CCXXIV).

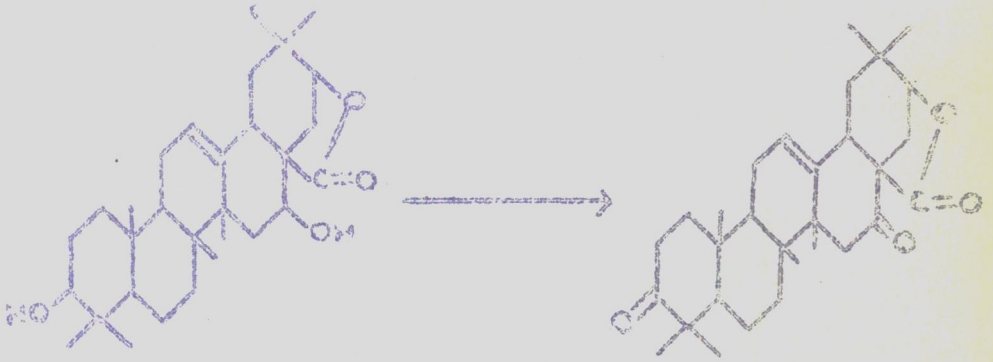


(Fig. X)

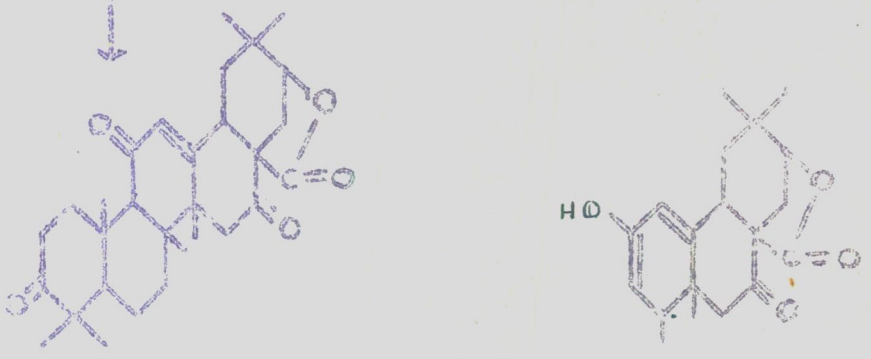


(XXXI)

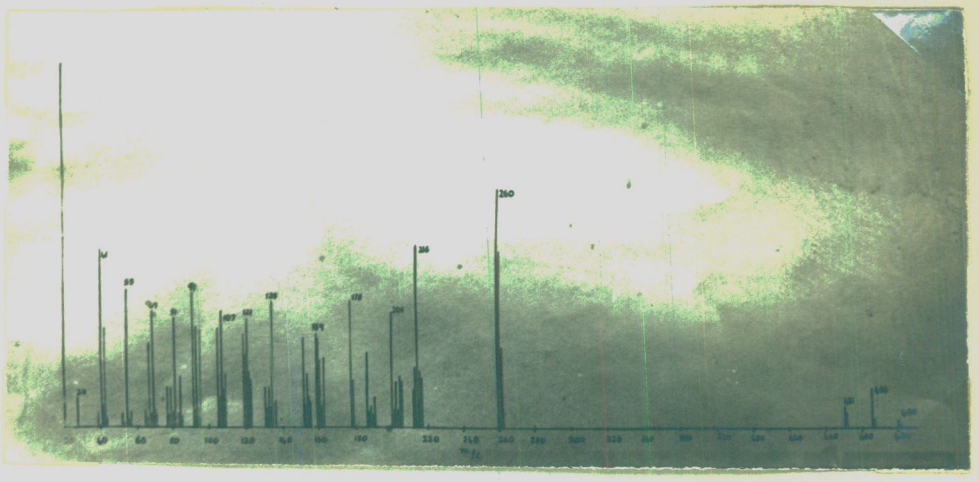
The spot of the 11-keto compound was very faint showing its presence in traces only. A weak peak at m/e 315 in the mass spectrum can be attributed to the ion (CCXXIX) arising out of the 11-keto compound (CCXXVIII).



(CCXXVII) (CCXXIV)

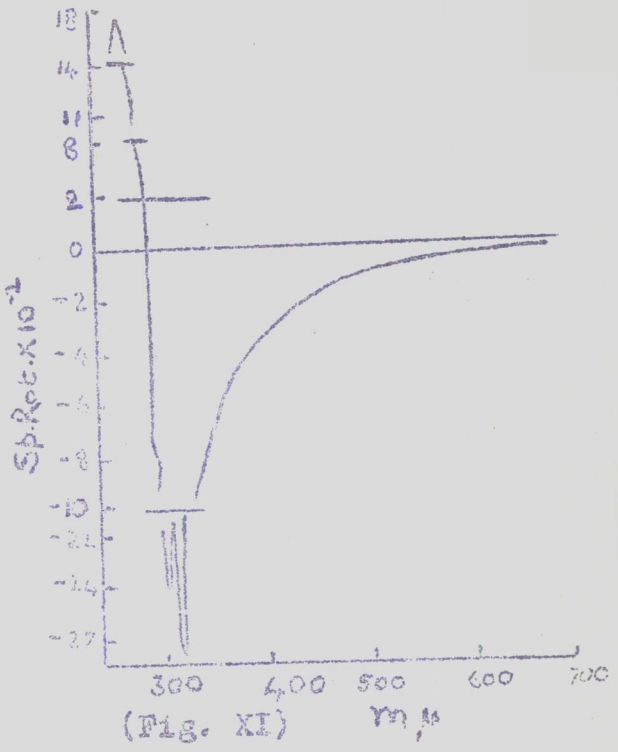


(CCXXVIII) (CCXXIX)



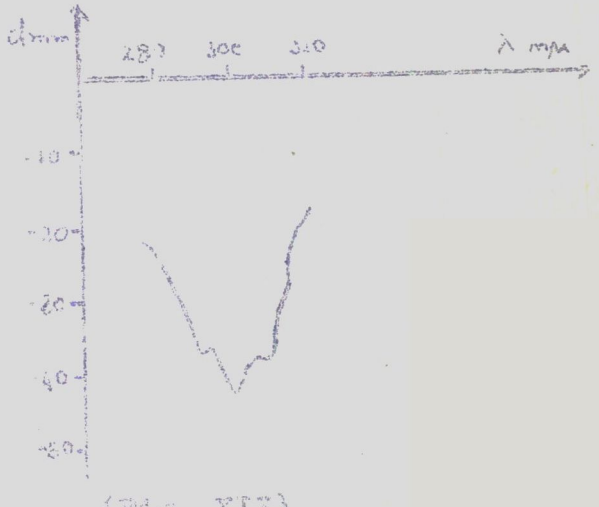
(Fig. IX)

The O.R.D. curve of this compound (Fig. X) shows a strong negative multiple cotton effect. This curve bears a strong resemblance to the O.R.D. curve of nor-ecdysterone acetate (CCXXX) (Fig. XI), which thus indicates that this compound (CCXXIV) carries a keto function at position 16 and that D-E ring junction is of the same type in both the compounds. These data definitely confirms the structure of this compound as a diketo lactone (CCXXIV).



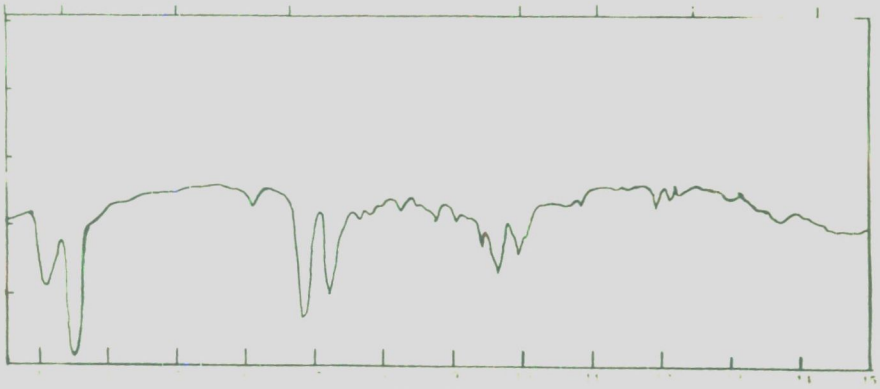
(Fig. XI)

The circular dichroism curve of this compound (Fig. XII) also is quite in agreement with the structure of the diketo lactone (CVIXIV).



(Fig. XII)

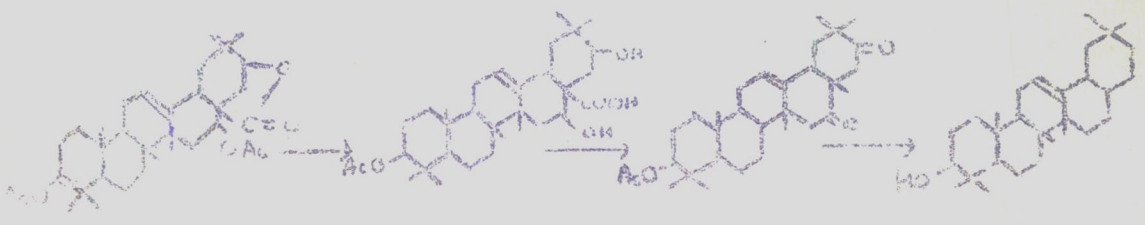
The ketone (CCXXIV) was subjected to Wolff-Kishner reduction[223,224] under forcing conditions. The product was crystallised from methanol:ethyl acetate, m.p. 183-88°. It gave a positive colour with tetranitromethane. The infrared spectra of this compound (Fig. XIII) shows a strong band in the hydroxyl region.

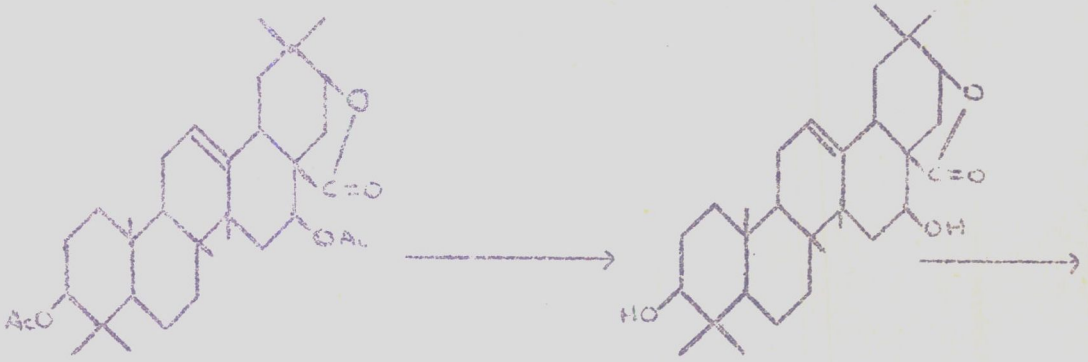


(Fig. XIII)

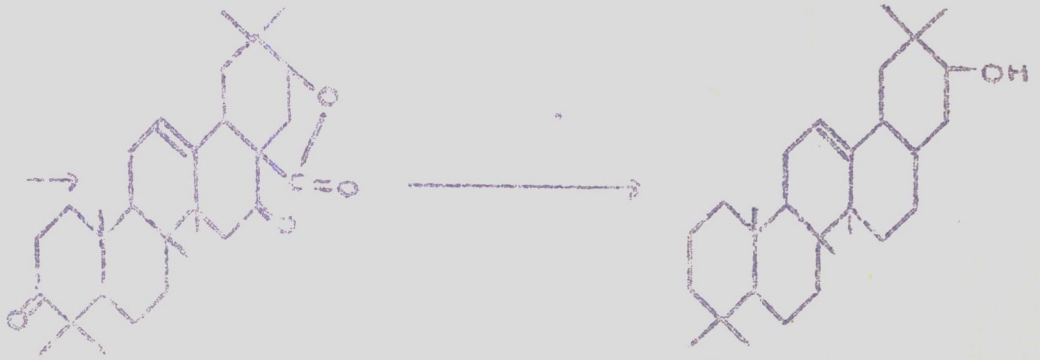
The analytical results corresponded with the formula $C_{29}H_{49}O$ and thus this compound should have the structure (CCXXXI)* on the basis of the scheme outlined.

* The product (CCXXI) was earlier thought to be 28-nor-β-amyrin according to the scheme of reactions shown but later infrared studies showed that this compound was different from 28-nor-β-amyrin.





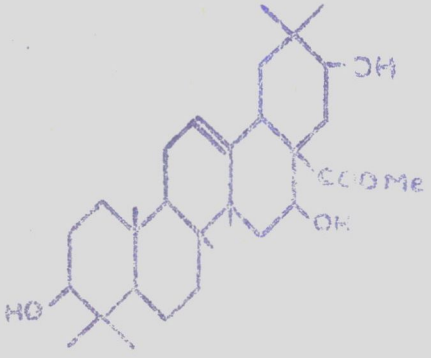
(CCXVII) (CCXVIII)



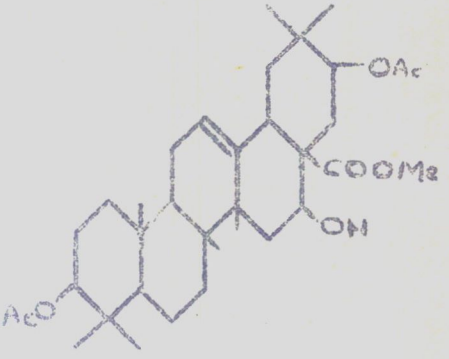
(CCXXIV) (CCXXXI)

The formation of the nor-compound starting with C-30 ketone (CCXXIV) definitely showed that the ketone was a β-ketolactone which can only explain the decarboxylation during the Wolff-Kishner reduction, due to the formation of a β-keto acid under the alkaline conditions of the reaction. This nor-compound (CCXXXI) formed an acetate with acetic anhydride and pyridine in the cold, m.p. 210-16°.

The formation of a diacetyl monohydroxy methyl ester (CCXXXIII) on acetylation of the methyl ester (CCXXXII) opened the possibility of preferentially manipulating one hydroxyl group. Thus the acetyl methyl ester (CCXXIII) was



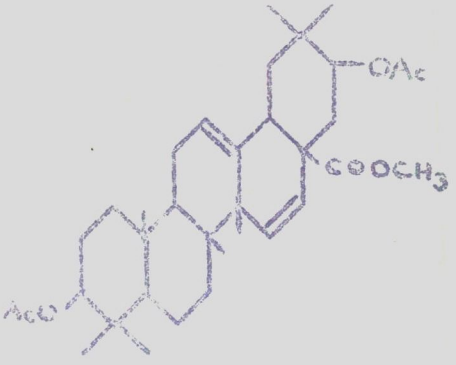
(CCXXXII)



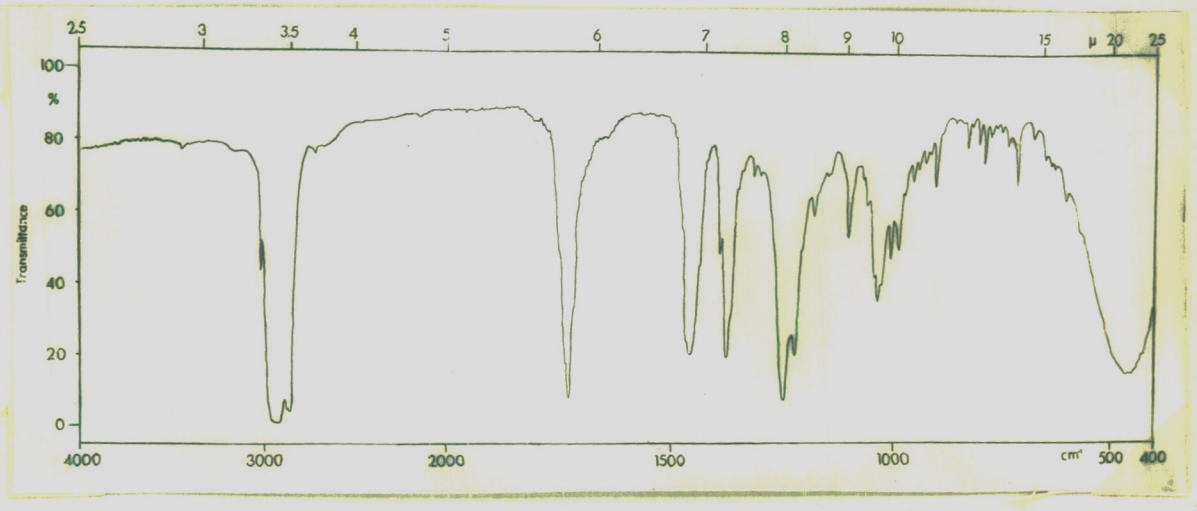
(CCXXXIII)

dehydrated with phosphorous oxychloride and pyridine. The product crystallised from methanol as shining needles m.p. 280-82°. It gave a strong yellow colour with tetranitromethane indicating the formation of an additional double bond, as the starting material gave only a light yellow colour. The infrared spectra of this compound (Fig. XIV) confirmed this postulation as the hydroxyl band disappeared after this reaction and this compound can be represented by the formula (CCXXXIV). This formulation derived support from the results of the elemental analysis of the compound, wherein it

corresponded to the formula $C_{35}H_{52}O_6$.

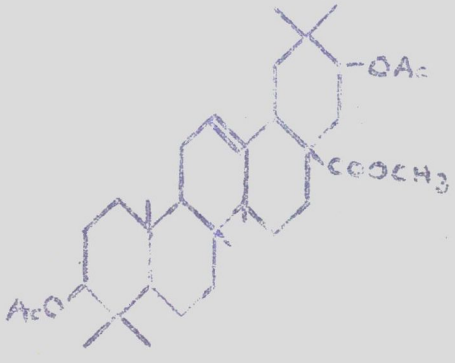


(OCXXXIV)

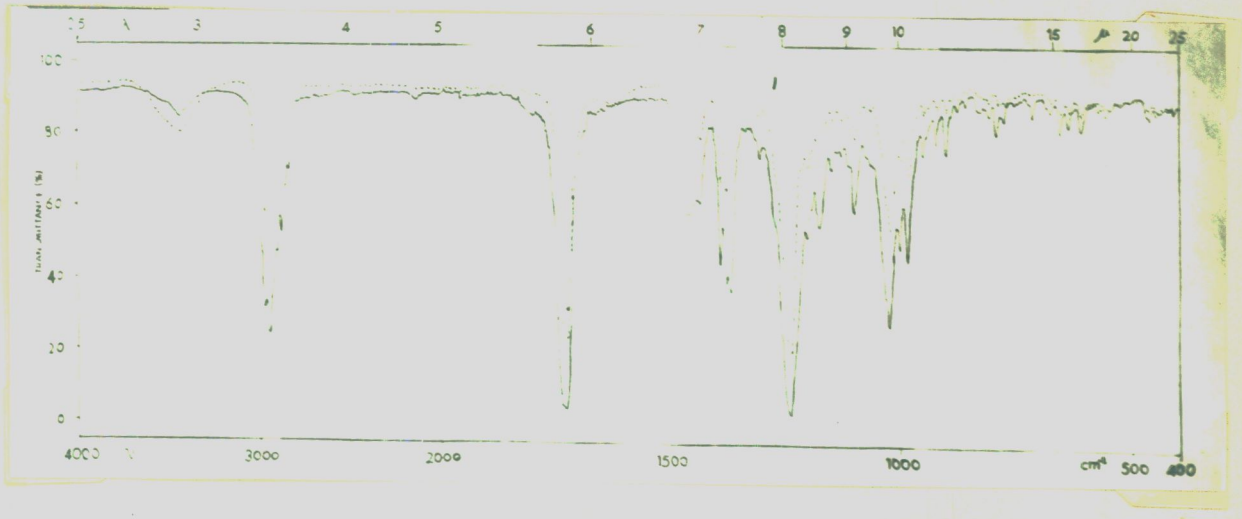


(Fig. XIV)

The diene (CCXXIV) obtained by the dehydration of the acetyl methyl ester was then hydrogenated in acetic acid solution in the presence of Adam's platinum oxide catalyst for four hours. The resultant product on crystallisation from methanol gave shining needles, m.p. 264-68°. The analytical results corresponded to the formula $C_{35}H_{54}O_6$. This composition was in agreement with the diacetyl methyl ester of machaerinic acid or its isomer proceric acid (CCXXXV)[203], but as its melting point was in nether agreement with that of the acetyl methyl ester of proceric acid a mixed melting point was taken with a sample of acetyl methyl ester of proceric acid[203] and no depression was observed. Further the infrared spectra of both the compounds were superimposable (Fig. XV). Therefore this product has been identified as the diacetyl methyl ester of proceric acid (CCXXXV) (diacetyl methyl ester of 3,21 dihydroxy olean-12-ene-28-oic acid).

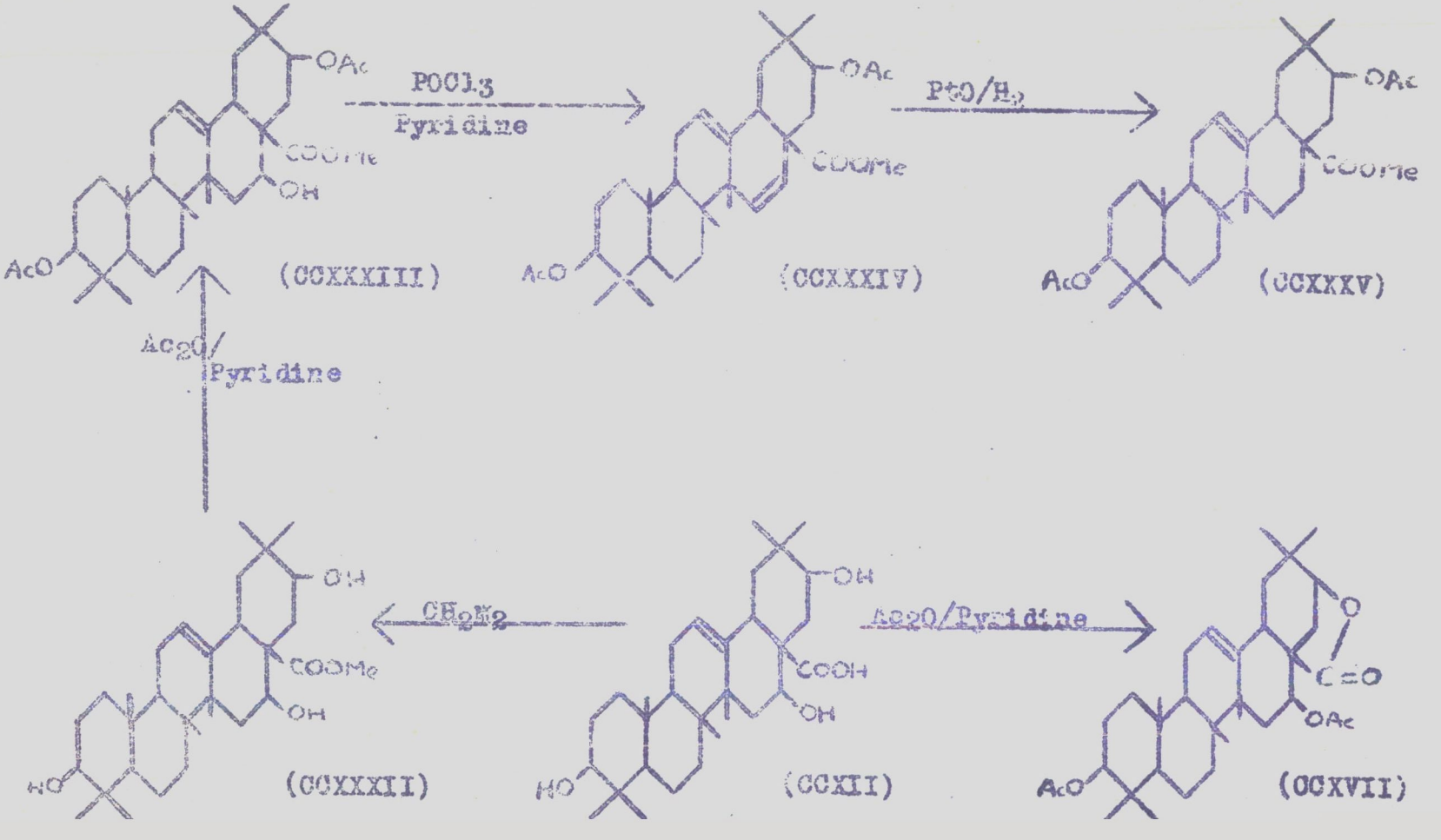


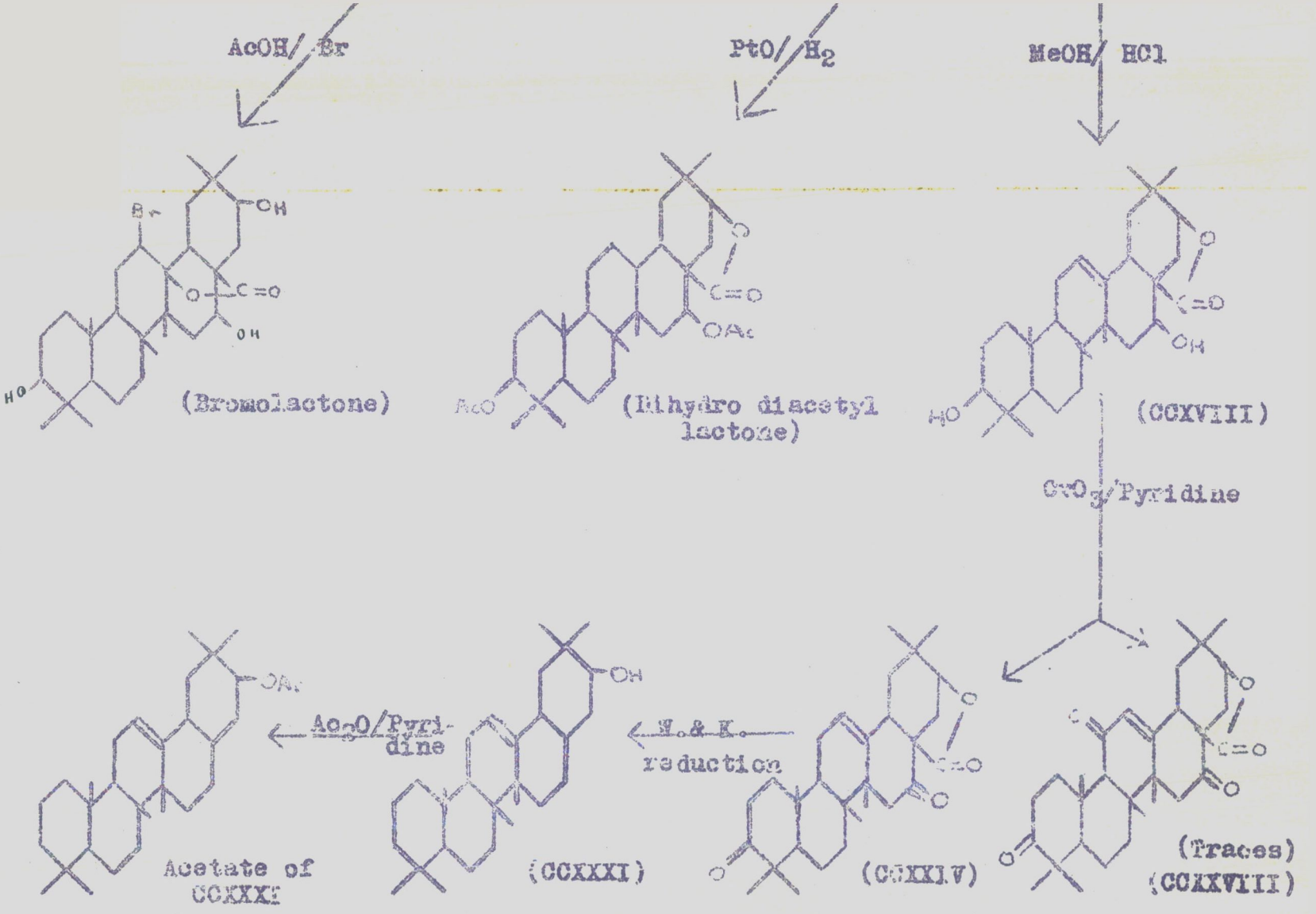
(CCXXXV)



(Fig. XV)

The transformations effected in this work and on which the structure of acacic acid is based can be summarised as follows:


OAc
COOMe
OH
AcO
(CCXXXIII)
POCl3
Pyridine
(CCXXXIV)
Pt0/H2
(CCXXXV)
Ac2O/
Pyridine
CH2N2
(CCXXXII)
COOH
(CCXII)
Ac2O/Pyridine
O
C=O
(CCXVII)
HO


AcOH/ Br
PtO/ H2
MeOH/ HCl
(Bromolactone)
(Dihydro diacetyl lactone)
(CCXVIII)
CrO3/Pyridine
Ac2O/Pyridine
W. & K. reduction
Acetate of CCXXXI
(CCXXXI)
(CCXXIV)
(Traces)
(CCXXVIII)

The formation of the bromolactone in conjunction with findings of the Infrared, NMR and Mass spectra (including fragmentation scheme), O.R.D. and Circular dichroism studies proved conclusively that Acacic acid belongs to the β-amyrin group.

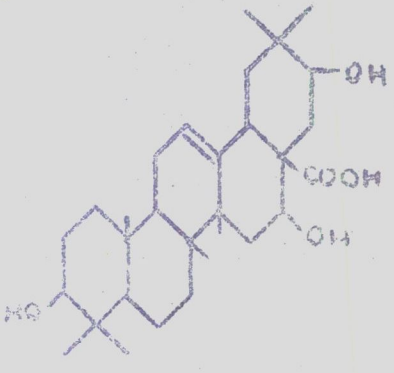
The position of the carboxyl group at C-17 has been proved by the formation of the bromolactone as well as by the smooth catalytic reduction of the double bond in the diacetyl lactone of acacic acid (CCXVII) in confirmity of the findings of Djerassi et. al.[216]

One hydroxyl has been fixed at the ubiquitous 3-position as found in almost all the triterpenes of the β-amyrin group, which is further confirmed by the production of acetyl methyl ester of procaric acid (CCXXV) from the acetyl methyl ester of acsoic acid (CCXXIII), which also fixes the position of second hydroxyl group at C-21.

The third hydroxyl group has been fixed at position 16 as the Wolff-Kishner reduction of the diketolactone (CCXXIV) formed by the oxidation of the dihydroxy lactone (CCXVIII) leads to a nor-compound (CCXXXI) due to the decarboxylation of a β-keto acid formed under the reaction conditions.

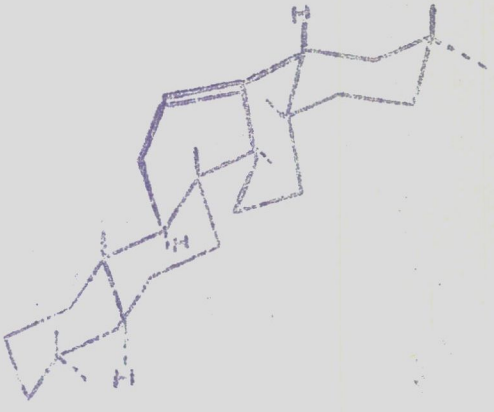
Of the two possible positions γ to the carboxyl group; C-16 and C-22, the position C-22 has been outruled as the methyl ester of acacic acid (CCXXII) failed to undergo periodic acid oxidation and also as it did not form an isopropylidene derivative. The position of the third hydroxyl as C-16 was further substantiated by the O.R.D. values (Fig. X) of the diketolactone (CCXXIV) which showed a strong negative multiple cotton effect, completely resembling the O.R.D. curve of norchinacystenalone acetate (CCXX Fig. XI).

All these evidences obtained by degradative studies in conjunction with the infrared, NMR, and Mass spectrographic studies and the studies of O.R.D. and Circular dichroism curves proved that acacic acid is 3,16,21-trihydroxy olean-12-ene-oic acid. (CCXII).



(CCXII)

Once the structure of acacic acid was definitely concluded to be (CCXII) the stereochemistry and conformations remained to be fixed. Usually the triterpenes belonging to the β-amyrin group have the rings A-B, B-C and C-D trans-fused and the rings D-E cis-fused, with an all chair conformation (CCXXXVI).

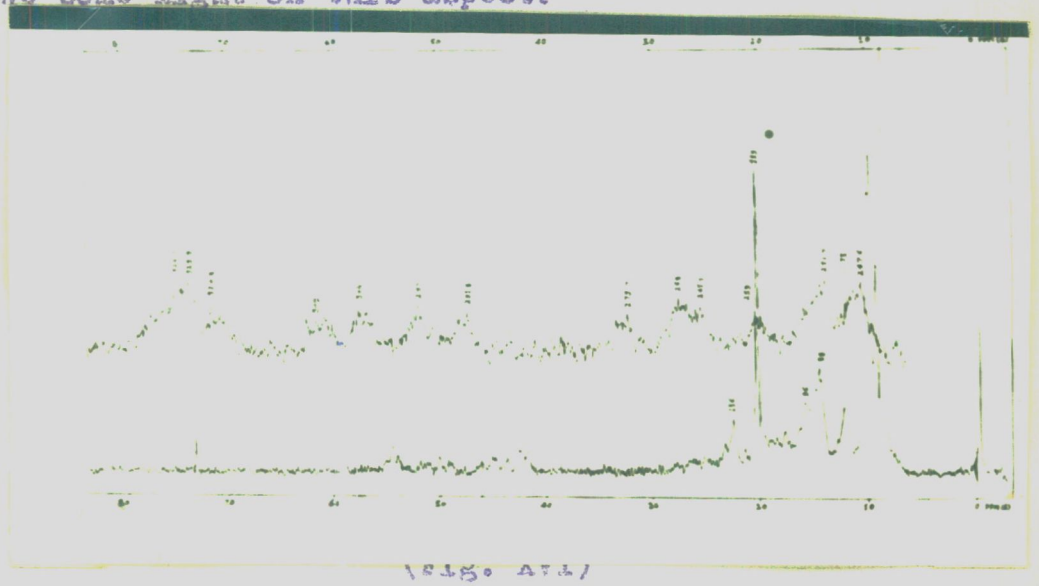


(CCXXXVI)

The easy lactonisation of acacic acid between the 21-hydroxyl and the 28-carboxyl on acetylation, even in the cold, cannot be explained if this arrangement is assigned to acacic acid. Further the easy hydrogenation of the diacetyl lactone (CCXVIX) in presence of platinum oxide catalyst is contrary to the fact that the double bond of the members of the β-amyrin

group do not hydrogenate under the normal conditions [78,216] and indicate that some subtle conformational differences exist in this compound. This instance is parallel to the behaviour of the sapogenin B of Styphnodendron coriaceum (CCXVI) which also hydrogenates easily. This abnormal behaviour can be explained on the basis that the ring E exists in boat form and ring D in quasi-boat form, Cf: Djerassi et al [216]. Therefore it can be assumed that in acacic acid rings D and E exist in quasi-boat and boat forms respectively which could explain the easy lactonisation. The proximity of the C-23 hydroxyl and the C-28 carboxyl groups which tends to make the lactonisation easy can be seen on the study of a molecular model of acacic acid. Such a lactonisation could also be possible with a trans D-E fusion but this possibility is eliminated on the basis of the O.R.D. curve of the diketolactone (CCXXIV, Fig. XI) which shows a strong negative multiple cotton effect, strongly resembling that of nor-echinocystenalone acetate (CCXXX, Fig. XI), which also has a D-E cis fusion. This resemblance of the O.R.D. curves indicate identical stereochemical arrangements in both the compounds, around ring D and thus definitely proves that the D-E ring junction is cis in nature, for a trans fusion should be expected to lead to a positive cotton effect.

This leaves the orientations of the three hydroxyl groups; 3,16 and 21 to be determined in acacic acid. The NMR spectra of acacic acid diacetyl lactone (COXVII, Fig. XVI) throws some light on this aspect.



(Fig. XVI)

The NMR spectra (Fig. XVI) showed signals corresponding to the lactonic proton at 4.2 ppm. as a doublet, This is in quite agreement with the structure formulated, for the dihydral angles between the hydrogen at C-21 and one of the hydrogens at C-22 is about 90^{o} which leads to a doublet rather than the triplet normally obtained by splitting of vicinal hydrogens. The acetoxyl proton on C-3 appears in the form of a quadrapulet centered at 4.5 ppm; J_1 = 9 cps and J_2 = 2 cps, which indicates

that the hydrogen is α and the hydroxyl β oriented. The other acetoxyl proton of C-16 appears in the form of a quadrapulet centered at 5.0 ppm. J_1 = 12 cps J_2 = 5 cps, also indicating a β orientation for the hydroxyl group. The hydroxyl on C-21 also, on the basis of lactone formation should be designated β. This indicates that all the three hydroxyl groups in this compound are β -oriented.

Therefore Acacic acid is 3β,16β,21β-trihydroxy olean-12-one-18β-28 oic acid with rings E and D in boat and quasi-boat forms respectively, with a D-E cis fusion (CCXXXVII).

(CCXXXVII)

2. Albizzia amara Bentb.

Albizzia amara Bentb is a member of the family Leguminoseae, sub-family Mimoseae. It is a middle sized deciduous tree, resembling othe Albizzia plants. The oil of the seeds of this plant is given in white leprosy and the flowers and the leaves are applied in local inflamations such as boils, erysipelas etc. The seeds are astringent, given in piles, diarrhoea, and gonorrhoea. Flowers are considered as a cooling medicine and are also externally applied to boils, eruptions and swellings. Leaves are reported as useful in opthalmia and afford good food for cattle.

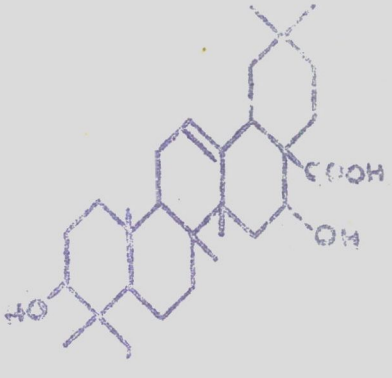
Although much work has been reported on other species of Albizzia, no reference is available in the literature on the seeds of Albizzia amara and therefore the study of the saponin and sapogenin contents of this plant was undertaken.

Well powdered seeds of Albizzia amara obtained from Messrs Johnson Sons & Co., Alleppey, Kerala were defatted with light petroleum ether. The seed powder was then exhausted with ethanol and the saponin purified and worked out in the usual manner. The saponin thus obtained was hydrolysed with sulphuric acid (10%) in the usual manner. The genin obtained was filtered, washed free of acid and purified by sodium salt formation. The sodium salt was decomposed with hydrochloric acid. The acid genin thus obtained could not be crystallised and therefore was converted into acetate with

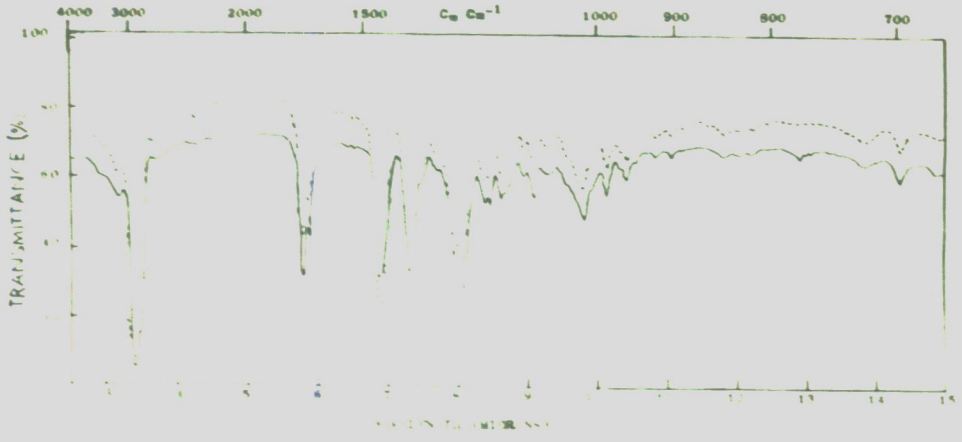
pyridine and acetic anhydride in the cold in the usual manner. The acetate on repeated crystallisations from methanol gave colourless needles, m.p. 255-60°.

The acetate m.p. 255-60° was readily transformed into a methyl ester by diazomethane and on crystallisation from methanol had m.p. 201-202°. Both the derivatives gave usual tests for triterpenes and positive Liebermann-Burchard and tetranitromethane tests.

The melting points of these derivatives were in good agreement with the corresponding derivatives of echinocystic acid and therefore a mixed melting point with the corresponding derivatives of authentic sample of echinocystic acid[201], was taken which confirmed this acid to be echinocystic acid (CCXXXVIII). The acid genin from Albizzia amara was further confirmed as echinocystic acid by superimposable infrared spectra of their acetates (Fig. XVII).



(CCXXXVIII)



(Fig. XVII)

3. Pithecolobium dulce Benth (Ing; dulcis Willd):

Pithecolobium dulce Benth (Ing; dulcis Willd) belongs to the family Leguminoseae, sub-family Mimoseae. It is a large thorny tree found throughout the plains of India and is largely grown in South India for use as fuel. The thick twisted pods contain a quantity of sweet whitish pulp, which is eaten. The seeds contain a large amount of oil.[226]

A review to the literature showed that no work on the saponin contents on this plant has been done and hence a study of the seeds of this plant was taken up. Towards the end of the work on these seeds Mitra et al[227] reported the isolation of the following products from this plant. The seeds were reported to contain a saponin, m.p. 175-81°; sapogenin, pithogenin ($C_{28}H_{44}O_4$) m.p. 207-8°, $(\alpha)_D$ +81; a sterol glycoside B, m.p. 278-80°; aglycone, m.p. 259-60° and a flavone, m.p. 209-303°. The mesocarp of this plant was reported to contain a sterol glycoside, m.p. 282-86°; aglycone m.p. 136-38° and certain amino acids. But our results completely differ from the results obtained by Mitra et al.[227]

The seeds of Pithecolobium dulce Benth obtained from Messrs Johnson Sons & Co., Allepey, Kerala were defatted and then exhausted with ethanol. The recovery of the solvent

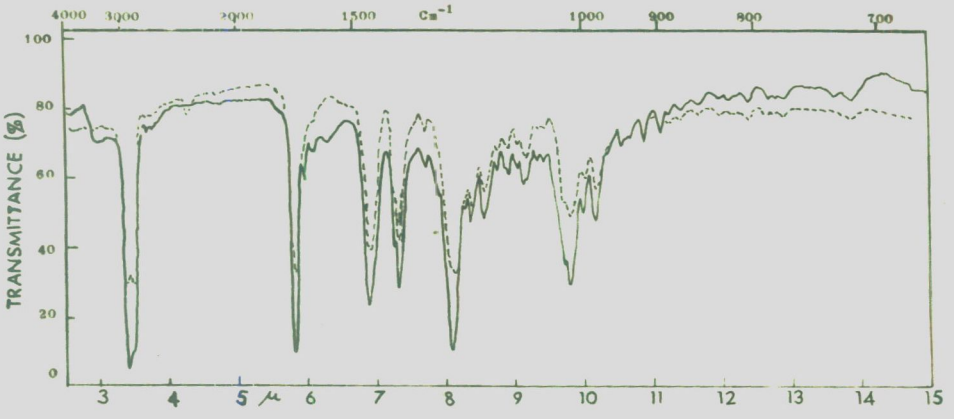
left a gummy mass which was treated in the usual manner as applied to saponins. The saponin obtained by several precipitations with ether/acetone gave a moderately pure specimen of saponin which gave all the tests for it.

The saponin thus obtained was dissolved in a large volume of water and hydrolysed by sulphuric acid (10%) by heating on a water bath followed by refluxing. The genin which separated was washed free of acid. It was then purified by the formation of sodium salt by refluxing with an alcoholic solution of sodium hydroxide and extraction of the aqueous solution with ether. Evaporation of the ethereal layer gave a neutral fraction. The alkaline solution was acidified with hydrochloric acid and the precipitated acid genin was unsuccessfully crystallised from various solvents and ultimately it was acetylated in the cold with pyridine and acetic anhydride. The acetate on repeated crystallisations from methanol gave two products A, m.p. 286-88° and B, m.p. 215-22°.

The two acetates were methylated with diazomethane. The methyl esters from the acetates A and B had melting points 269-74° and 217-22° respectively.

The acetate m.p. 286-88°; acetate methyl ester m.p. 269-74° has been identified as procaric acid, earlier isolated from the seeds of Albizzia procera Benth from Maharashtra, by mixed melting point of the acetate methyl ester with an authentic sample of acetate methyl procerate and super-

impossible infrared spectra (Fig. XVIII).



(Fig. XVIII)

The product B acetate m.p. 215-22° acetate methyl ester m.p. 217-22° gave a positive reaction with tetranitromethane for double bond. It also gave a positive reaction with the arsenic chloride reagent indicative of the fact that it is a triterpene.

4. Sesbania speciosa Taub:

Sesbania speciosa Taub belongs to the family Leguminoseae; sub-family Papilionaceae. It resembles the other species of Sesbania in character. A review of the literature showed that no work has been done on the seeds of Sesbania speciosa Taub, whereas three other species of Sesbania have been studied for their saponin and sapogenin contents. Sesbania aegyptica[214] and Sesbania aculeata[215] have been reported to contain oleanolic acid in the form of saponin and a neutral product different from each other. The leaves of Sesbania grandiflora[223] have been reported to contain only oleanolic acid. Therefore the study of the seeds of this plant was taken up.

The seeds of Sesbania speciosa were obtained from Messrs Johnson, Sons & Co., Allepey, Kerala. A quantity of the finely powdered and defatted seeds were exhausted with ethanol. The recovery of the solvent left a brownish residue which was successively treated with ether, petroleum ether, carbontetrachloride and acetone and the saponin obtained in the usual manner by precipitation with ether/acetone. The saponin obtained was dissolved in a large amount of water and hydrolysed with sulphuric acid (10%) in the usual manner. The genin obtained was filtered, washed free of acid and dried. The crude genin was then refluxed with an alcoholic solution of potassium hydroxide for two hours, concentrated to half the volume and diluted with water. The diluted

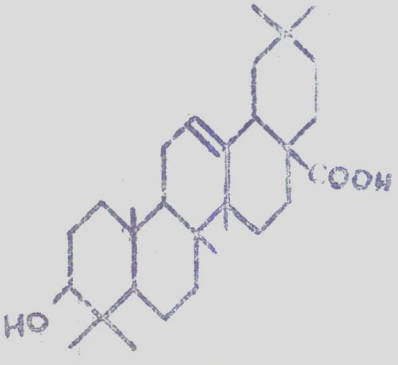
solution was extracted with ether. The ether extract was washed free of alkali and the ether recovered to yield the neutral fraction. The alkaline aqueous layer on acidification with hydrochloric acid precipitated the acid genin which was filtered, washed free of acid and dried.

The acid genin obtained was acetylated in the hot with acetic anhydride and pyridine and the product worked out in the usual manner. Repeated crystallisations from methanol yielded fine needles m.p. 260-64°. It gave a positive colour with tetranitromethane. A part of the genin was also converted into the methyl ester with diazomethane. The product on crystallisation from methanol finally had m.p. 196-98°. The methyl ester also gave a positive reaction with tetranitromethane. Both the derivatives gave positive reactions with the stannic chloride reagent. As the melting points of both the derivatives resembled the melting points of the corresponding derivatives of oleanolic acid, mixed melting points of the acetate and methyl ester of the isolated genin and the respective derivatives of oleanolic acid was taken, and no depression observed in either case.

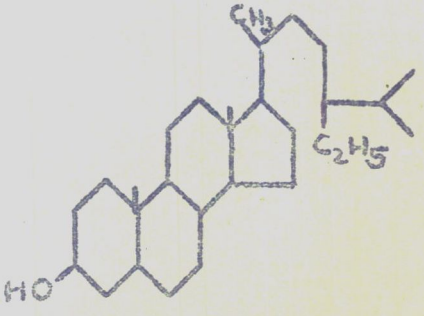
Attempts to crystallise the neutral fraction from various solvents were unsuccessful and hence it was chromatographed on alumina. The chromatography yielded two products m.p. 145-50° and m.p. 252-53°. The acetates of both the products were prepared with acetic anhydride in the cold and

worked out in the usual manner. The product 145-50° gave an acetate m.p. 132-36°. It gave no colour with tetranitromethane. As the melting points of these derivatives were found to be in close agreement with the melting points of the corresponding derivatives of β-sitostanol, mixed melting points were taken with the respective samples obtained through the courtesy of Prof. Itsuo Nishioka, University of Kyushu, Japan and no depression was observed. The second neutral product m.p. 252-58° gave a positive colour with tetranitromethane and also a positive reaction with stannic chloride for triterpenes. Its acetate crystallised from methanol, m.p. 162-68°. This neutral portion is different from those earlier isolated from two other species of Sesbania; Sesbania aegyptica and Sesbania aculata.

Thus it has been concluded that the seeds of Sesbania speciosa contain oleanolic acid (CCXXXIX), β-sitostanol (CCXL) and an unidentified neutral triterpene m.p. 252-58°; acetate m.p. 162-68°.



(CCXXXIX)



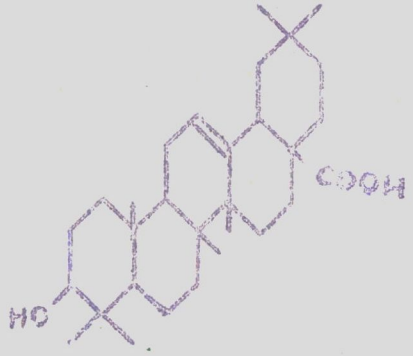
(CCXL)

5. Psidium guaijava Linn:

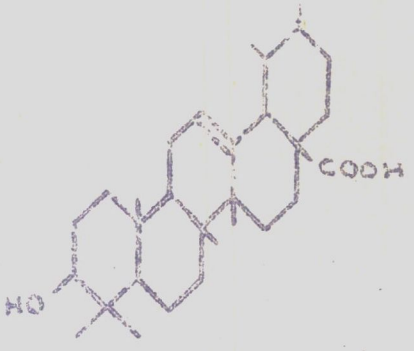
Psidium guaijava Linn belongs to the family Myrtaceae. It is a small evergreen tree or large shrub about 20-30 feet high with a girth of 2-3 feet and is cultivated throughout India. It is commonly known as gauva (amrood in Hindustani) and its fruits is eaten. The leaves when chewed are said to be a remedy in tooth-ache.[229] Locally decoctions of the leaves are applied with much benefit to the prolapsus ani of children, in scurvy and for unhealthy ulcers and is an efficacious gargle for swollen gums and ulceration in the mouth. Leaves when ground make excellent poultice.[230]

The leaves of Psidium guaijava Linn from Egypt have earlier been studied and found to contain a new triterpenic acid, psidiolic acid.[231] Later on Arthur and Hui[232] reinvestigated the leaves from Hong Kong and have reported that it contains ursolic acid (CCXLI), oleanolic acid (CCXXXIX), oraetagolic acid (maslinic acid) (CCXLII) and a new triterpenic acid named as guaijavolic acid. Guava is a fruit which is very common and eaten throughout India, but no work seems to have been done on the leaves of this plant from Uttar Pradesh. Being ourselves engaged on the study of saponins and sapogenins[195] it seemed of interest to study these leaves from Uttar Pradesh as it has now been reported that for the same plant the saponin and sapogenin content vary not only in the percentage content but also in their nature and therefore it

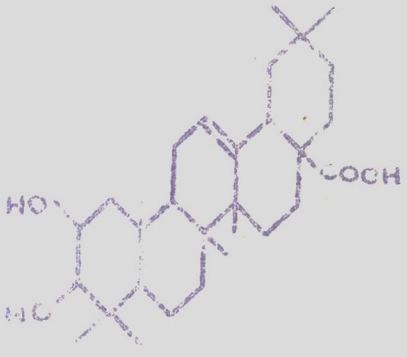
will not be unnatural if the constituents of the leaves from Uttar Pradesh differ from those of Hong Kong studied earlier and therefore the study of the leaves from Uttar Pradesh was taken up.



(CCXXXIX)



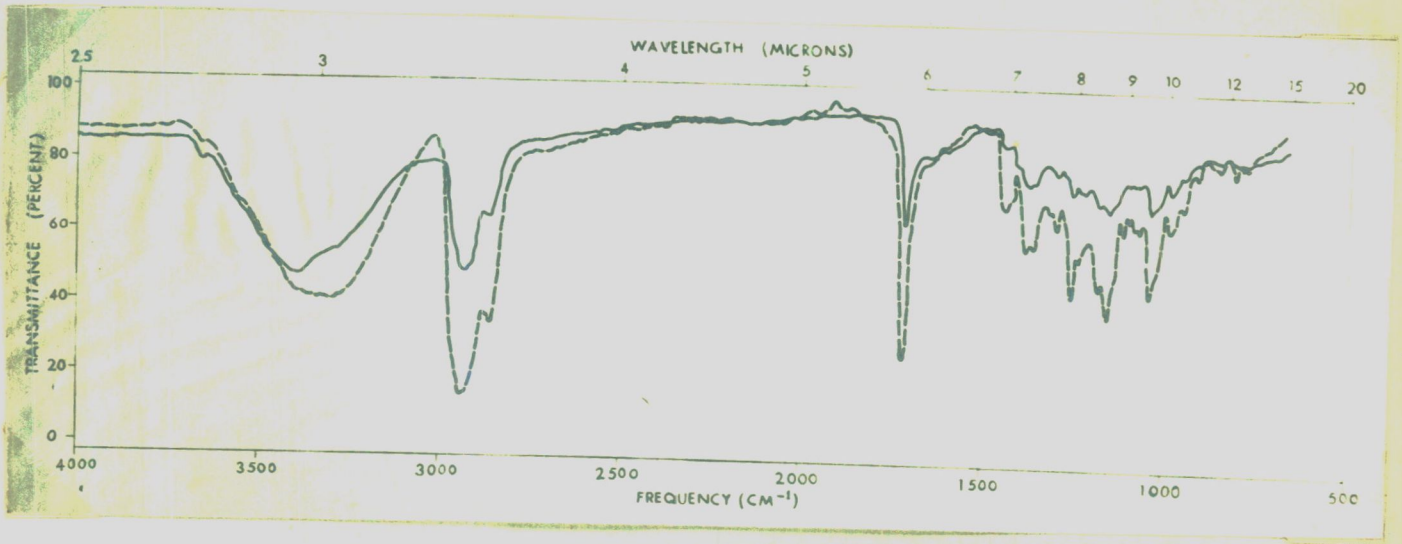
(CCXLI)



(CCXLII)

on alumina using benzene, benzene-ether and finally ether as eluants. In total 40 fractions were collected. The fractions 5-8 (benzene) gave a product 'A' m.p. 102-104°, the fractions 22-24 (ether:benzene; 1:1) gave a product B m.p. 212-14° and the fractions 26-30 (ether:benzene; 1:1) yielded a product 'C' m.p. 220-22°, which is the major product.

The product 'B' m.p. 212-14° on rechromatography on alumina was resolved into two fractions and one of the two fractions m.p. 205-207° was termed as product 'D'. The second fraction m.p. 220-22° was found to be identical with the product 'C' m.p. 220-22°, which is an ester has been identified as oleanolic acid (maslinic acid) (CCXLII) by superimposable infrared spectra obtained through the courtesy of Prof. R. Tschesche of the University of Bonn. (Fig. XIX)



(Fig. XIX)

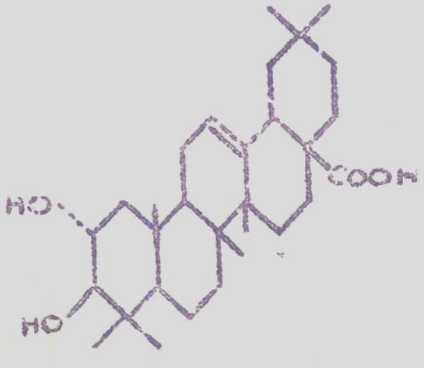
The green leaves collected locally from the campus, were exhausted with ether in the cold and the recovery of the solvents resulted in the isolation of a greenish gummy mass which was treated with alkali and extracted with ether. The ethereal layer on recovery yielded a neutral product which was unsuccessfully crystallised from various solvents. The chromatography on alumina and elution with benzene gave some oily product and two colourless crystalline products m.p. 80-82° and 137-40°. The product m.p. 137-40°; acetate m.p. 128-29° was identified as β-sitosterol by mixed melting point with an authentic sample of β-sitosterol. Further elution of the chromatogram with benzene:acetone (1:1) gave another colourless neutral product m.p. 153-55°. It gave a positive reaction for triterpene and also for double bond with tetranitromethane.

The alkaline aqueous layer left after ether extraction was acidified with hydrochloric acid, which precipitated the acid fraction. All attempts to crystallise it were unsuccessful and therefore the genin was transformed into methyl ester by diazomethane and had m.p. 168-72°. The methyl ester gave an acetate methyl ester m.p. 236-38° and the genin on acetylation gave an acetate m.p. 225-31°. It gave all tests for triterpenes. However as the melting points of these derivatives were not sharp this acid seemed to be a mixture and therefore the methyl ester was chromatographed

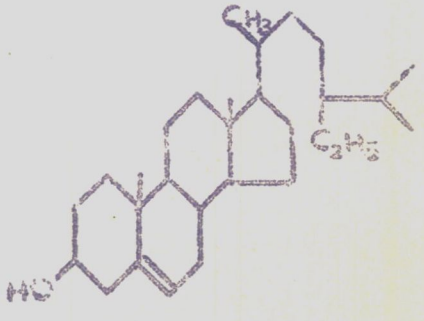
The product 'D' m.p. 205-207° has been identified as guaijavolic acid[232] by mixed melting point with an authentic sample of methyl guaijavolate obtained through the courtesy of Dr. H.R.Arthur of the University of Hong Kong.

All attempts to isolate ursolic acid and oleanolic acid reported by the earlier authors[232] were unsuccessful and therefore it has been concluded that the leaves of Psidium guajava Linn from Uttar Pradesh, India, does not contain any of these products.

Therefore it is concluded that the leaves of Psidium guajava from Uttar Pradesh, India, contain β-sitosterol, (CCXLIII) crataegolic acid (maslinic acid) (CCXLII) guaijavolic acid, an unidentified acid whose methyl ester melts at 102-104° apart from a neutral triterpenic sapogenin m.p. 153-155° and another product m.p. 80-82°.



(CCXLII)



(CCXLIII)

6. Balanites roxburghii Planch:

Balanites roxburghii Planch syn. Balanites aegyptica Linn is a member of the family Simaroubaceae, known as "Hingota" in Hindustani. It is a small spiny tree about twenty feet high. The fruits are ovoid, about two inches in length. The stone encloses a oily seed. It is found in the northern parts of India and Burma. The pulp of the fruit is reported to be used for cleaning silk and cotton. The seeds, fruits bark and leaves are reported to be anthelmentic and purgative. In western India, the back is used as anthelmentic for cattle and its juice as fish poison in Panch Mahal, Bombay.[233] The seeds are given in coughs and colic and also in snake bite.[234]

The fruits, pulp and seed kernels of Balanites roxburghii are rich in saponin contents. The fruits of Balanites roxburghii are oval in shape about 15 gms. in weight consisting of thin outer covering followed by thick outer pulp, the removal of which gives the hard seeds. The seeds on breaking open by a hammer yields the white seed kernels.

A review to the literature showed that the pericarp of Balanites roxburghii have been studied for saponin contents and reported to contain diosgenin.[235] Later Kinol and Gedeon[236] reinvestigated the green fruits; probably the whole fruits and found that they contain diosgenin and kryptogenin. But it is regretted that no study of various parts separately has been done and it may be likely that different parts may

contain different genins. Recently Dutta studied the saponins from the pericarp and seed kernel separately and isolated two different saponins for which no melting points and analytical values are reported and have found that the saponin of the pericarp contains glucose and rhamnose and that of the seed kernel glucose, ribose, xylose and rhamnose as sugars. There is no mention of the nature of the genin or genins obtained from these two saponins. As no systematic study has been done on this plant it seemed desirable to reinvestigate various parts of the fruits of Balanites roxburghii systematically for the saponin content.

The fruits of Balanites roxburghii obtained from the Forest department (Uttar Pradesh) through the Silviculturist, U.P., Nainital, gave 7.8 per cent pericarp, 10 per cent pulp, 72.6 per cent seedcoat and 9.6 per cent seed kernels. The seed kernels yield 40 per cent golden yellow oil by extraction with light petroleum ether.

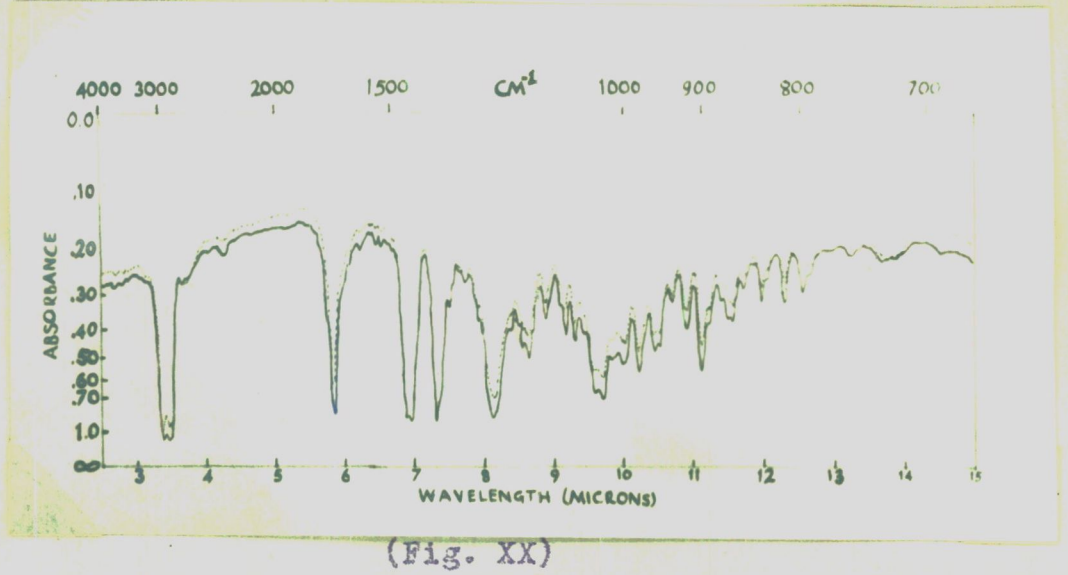
The well defatted seed powder was exhausted with ethanol. The recovery of the solvent gave a dark brown syrup, which was successively treated with petroleum ether, ether, carbon-tetrachloride, chloroform and acetone, to remove these solvent soluble impurities. The solid mass obtained by this treatment was dissolved in a minimum quantity of alcohol and the solution added to a large amount of ether/acetone dropwise, which precipitated the saponin. The process of dissolution and precipitation was repeated several times, when after

treatment with activated charcoal, a colourless nonhygroscopic powder of the saponin, m.p. 128-36° was obtained. It gave all tests for saponin.[4] Examination of the saponin with specific colour reagents indicated it to be steroidal in nature.

The saponin m.p. 128-36°, on chromatography on Whatman filter paper No. 1, using butanol:acetic acid:water (4:1:5) as solvent mixture and utilising both ascending and descending techniques, showed the presence of three fractions, one in major quantity and two in traces. The spray reagents utilised were Sannie's cinnamic aldehyde reagent[46,47] and para-dimethylaminobenzaldehyde reagent[48] of the steroidal saponins. The electrophoresis also confirmed these findings.

All attempts to purify the saponin by chromatography on various types of alumina, silicagel and cellulose were fruitless and therefore the purification of the saponin was done on prewashed Whatman filter paper No. 3 using butanol: acetic acid:water (4:1:5) as solvent system, employing the descending technique. The test strips were cut out of the developed chromatogram and the three fractions were located by spraying these strips with Sannie's cinnamic aldehyde reagent. The relevant portions were cut out and the saponin eluted with boiling alcohol. The alcoholic eluent from the major line spots was concentrated and the saponin precipitated with acetone, as a colourless powder m.p. 182-86°. It now showed only one spot on rechromatography.

The pure saponin m.p. 182-86° was hydrolysed with sulphuric acid (10%) and the sapogenin obtained was crystallised from acetone as colourless needles, m.p. 194-98°. The acetate of the sapogenin had m.p. 201-3° and the benzoate m.p. 233-36° The genin was identified as diosgenin by mixed melting points with authentic samples of diosgenin and acetate. This was further confirmed by the superimposable infrared spectra of the isolated genin acetate and an authentic sample of diosgenin acetate (Fig. XX). The saponin m.p. 182-86° has been named as Balanitesin.



(Fig. XX)

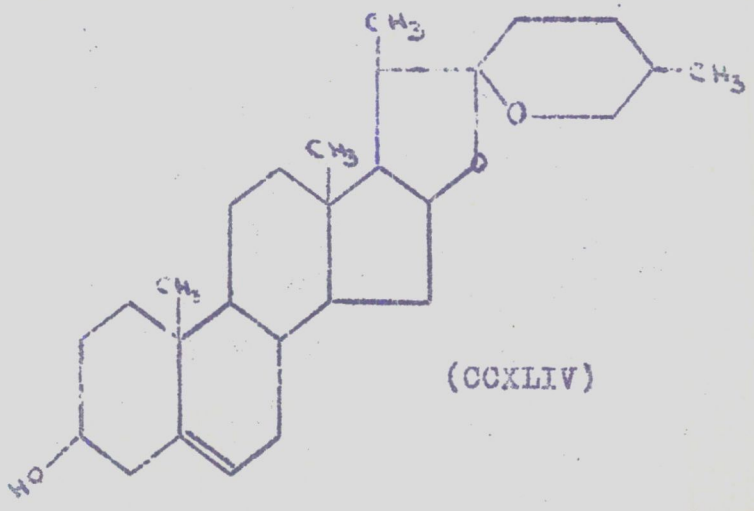
All attempts to isolate kryptogenin, earlier reported to be present as mixture with diosgenin in the whole fruits were unsuccessful. The crude saponin which was showing three spots, one major and two minor on chromatography, was

hydrolysed with sulphuric acid and the crude genin obtained was treated with Girard's P.reagent when a ketonic portion was obtained in traces (700 mg. of the crude genin gave only 10 mg of the ketonic part). All attempts to crystallise the ketonic part were unsuccessful and therefore it was transformed into an acetate. The acetate of the ketonic part was chromatographed alongside with an authentic sample of kryptogenin acetate on Whatman filter paper No. 1. After spraying with Sannié's cinnamic aldehyde reagent it was noted that not only the position of the spot of this ketonic part was different from that of kryptogenin acetate but also the colour. The position was also different from that of diosgenin acetate. Therefore it may be concluded that the seed kernels of Balanites roxburghii do not contain any kryptogenin earlier reported in the whole fruits of this plant and may be present in the other portions of this plant, but not definitely in the seed kernels.

In order to find out the nature of the sugar moieties in Balanitesin, the sulphuric acid hydrolysate of Balanitesin was neutralised with freshly precipitated barium carbonate, filtered and the precipitated barium sulphate washed with hot water. The filtrate and the washings containing the sugars

were evaporated to dryness in a vacuum oven at 35-40°. The concentrated sugar syrup was dissolved in a few drops of water and chromatographed on Whatman filter paper No. 1, along with authentic sugars using butanol:ethanol:water (4:11:19) as solvent mixture and utilising the descending technique. The spray reagent used was aniline hydrogen phthalate. To confirm the absence of keto-hexoses, urea HCl reagent was utilised. This showed the presence of the following three sugars; glucose, xylose and rhamnose.

Therefore Balanitesin has been found to be a new saponin of diosgenin (CCXLIV) with glucose, rhamnose and xylose as the sugar moieties.



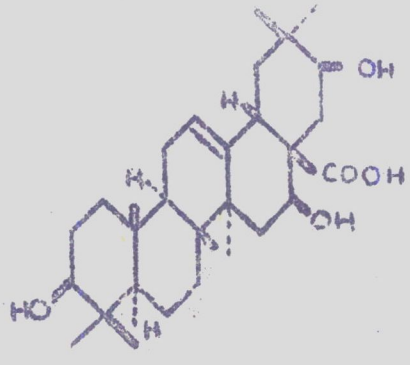
(CCXLIV)

C O N C L U S I O N S

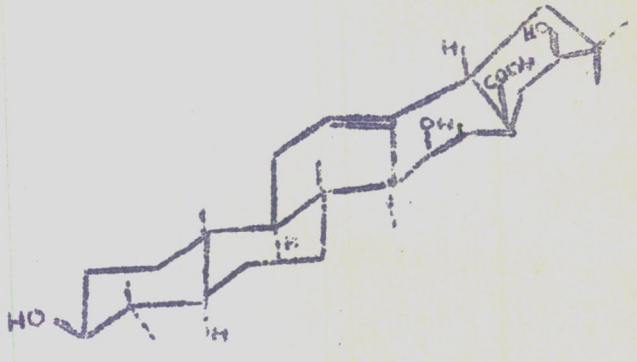
C O N C L I S I O N S

The following conclusions are drawn from this study.

1. The pods of Acacia concinna DC contain Acacic acid in the form of a saponin, Acacic acid m.p. 278-82°; diacetyl lactone m.p. 235-36°; methyl ester m.p. 223-24°; acetyl methyl ester m.p. 203-4°; Bromolactone m.p. 259-62°.
2. On the basis of degradative studies the structure of acacic acid has been fixed as 3,16,21-trihydroxy olean-12-ene-28-oic acid.
3. Acacic acid on the basis of IR, NMR and Mass (including fragmentational study) spectrographic studies and O.R.D. and circular dichroism studies has been fixed as 3β, 16β, 21β-trihydroxy olean-12-ene-18β-28-oic acid, with rings A and B in chair form D in quasi-boat form, E in boat form, and with A-B and B-C ring junctions trans fused and D-E ring junction cis fused. Acacic acid therefore can be represented by the following structures.



(I)



(II)

4. The seeds of Albizzia amara Benth from Kerala contain a triterpenic acid identified as echinocystic acid. It is present in the seeds in the form of a saponin.

5. The seeds of Pithecolobium dulce Benth. (Inga dulcis Willd.) from Kerala contain two triterpenic acid sapogenins; acetates m.p. 286-88° and 215-22°. The genin, acetate m.p. 286-88° has been identified as proceric acid.

6. The seeds of Sesbania speciosa Taub. from Kerala contain a triterpenic acid; oleanolic acid in the form saponin, in addition to β-sitosterol and an unidentified neutral triterpene, m.p. 252-58°, acetate, m.p. 162-68°.

7. The leaves of Psidium guajava Linn. from Uttar Pradesh contain crataegolic acid (maslinic acid), guaijavolic acid, an unidentified acid (methyl ester m.p. 102-4°), β-sitosterol, a hydrocarbon, m.p. 80-82° and a neutral triterpene m.p. 153-55°.

8. The seed kernels of Balanites roxburghii Planch. from Uttar Pradesh contain a mixture of three saponins, one in major and two in minor quantities. The major one has been obtained in pure form and named as Balanitesin. Balanitesin has been found to be a glycoside of diosgenin containing glucose, xylose and rhamnose as sugar moities.

A C K N O W L E D G E M E N T

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E X P E R I M E N T A L

E X P E R I M E N T A L

All the melting points recorded in this thesis have been taken on Kofler's hot microscopical stage and are corrected. The micro-analyses were carried out in this laboratory and at Laboratoire de Chimie, Museum National d' Histoire Naturelle, Paris, France.

The Infra-red spectra were taken in this laboratory, at National Chemical Laboratories, Poona (Perkin-Elmer Infracord, Model 137), at Organisch Chemischen Institut, der Universitat Bonn, West Germany (Perkin Elmer Model 221), at Laboratoire de Spectroscopie Moleculaire, Paris (Perkin Elmer Model 421) and at Indian Institute of Technology, Kanpur (Carl Zeiss Model U 126). The O.R.D. curve was recorded at the Chemistry Department, Stanford University, U.S.A. The NMR spectra, Mass spectra and the Circular dichroism curves were recorded through the courtesy of Prof. Guy Ourisson and Prof. R. Beyler of the Institut de Chimie, Strasbourg, France.

The electrophoresis was done on Jouan semi-automatic electrophoresis apparatus model No. 1603. The paper chromatography was carried out at room temperature.

Study of the pods of Acacia concinna DC

Extraction of the saponin:

The pods (900 g) broken into small pieces were extracted with ethanol thrice. On recovery of ethanol a dark brown syrup was obtained. It was successively treated with ether, petroleum ether, carbontetrachloride, chloroform and acetone. The mass left over was dissolved in a small quantity of alcohol and the saponin precipitated by addition to a large quantity of ether/acetone. This process of dissolution and precipitation was continued till a colourless specimen of the saponin was obtained.

Hydrolysis of the saponin:

The saponin (10 g) was dissolved in water and heated with sulphuric acid (10%) on a water bath for one hour and then refluxed for another hour. A brown precipitate was obtained which was filtered, washed free of acid and dried (yield 7.5 g).

Separation of the acid and neutral fractions:

The crude genin (7.5 g) was refluxed with alcoholic potassium hydroxide (10%, 600 cc) for an hour. Half of the solvent was recovered and the contents diluted with two litres of water. It was extracted with ether several times, the ethereal layers combined, washed free of alkali and ether recovered to yield a negligible amount of neutral portion.

The aqueous layer on acidification with hydrochloric acid precipitated the acid genin which was filtered, washed free of

acid, and dried (yield 3.2 g).

Acetylation of the acid genin:

(a) The crude genin (3 g) was refluxed with acetic anhydride (100 cc) containing a small quantity of fused sodium acetate, cooled and poured into ice cold water. The precipitate was filtered and washed free of acetic acid and crystallised from methanol; m.p. 235-36°. It gave a positive colour with tetranitromethane.

(b) The crude genin (5 g) was dissolved in pyridine (400 cc) and acetic anhydride (200 cc) added to it. The mixture was heated on a boiling water bath for two hours, cooled and poured into a large amount of iced water, with continous stirring. The precipitate obtained was filtered, washed free of pyridine and dried. It crystallised from methanol as needles, m.p. 235-36°. It gave a positive colour with tetranitromethane.

Deacetylation of the acetate:

The acetate (2 g) was refluxed with alcoholic potassium-hydroxide solution (300 cc; 10%) for two hours. The volume was reduced to half and the solution poured into excess of water. Acidification of the aqueous solution precipitated the genin which was filtered, washed free of acid and dried. It was crystallised from a large volume of methanol, m.p. 278-82°. It gave a positive colour reaction with tetranitro-methane and showed no depression on melting with an authentic

sample of acacic acid.

Methyl ester of the genin:

The genin (1 g) was suspended in ether (500 cc) and an excess of ethereal solution of diazomethane added to it. The reaction mixture was kept overnight at room temperature, after which the ether along with the excess of diazomethane were evaporated on a waterbath. The colourless residue left over was crystallised from methanol in fine colourless needles (1 g), m.p. 224-25°. It gave a positive colour with tetranitromethane. It showed no depression on melting with an authentic sample of acacic acid methyl ester.

Acetate methyl ester:

The methyl ester (1 g) was dissolved in pyridine (100 cc) and acetic anhydride (75 cc) added to it. The mixture was left overnight at room temperature and then poured dropwise into iced water with constant stirring. The precipitate formed was filtered washed free of pyridine and dried. It crystallised from methanol in the form of needles m.p. 203-05°. It gave a positive colour with tetranitromethane.

Analysis:

Found	C 71.46%	H 9.64%
	C 71.36%	H 9.50%
Calc.for $C_{35}H_{54}O_7$	C 71.64%	H 9.28%

Bromolactone of the acid gem:

Acacic acid (500 mg) was suspended in acetic acid (50 cc) containing anhydrous sodium acetate (2 g) and stirred with addition of bromine (1 cc) in acetic acid (25 cc). In about half hour the suspension dissolved. The stirring was continued for half hour more and then the solution poured into water. The product was extracted with ether and the ether extract washed with sodium thiosulphate solution, water sodium bicarbonate solution and water. The ether was recovered and the resultant product crystallised from methanol, m.p. 259-62°. The product gave no colour with tetranitromethane.

Attempted periodic oxidation of the methyl ester:

The methyl ester (500 mg) was dissolved in ethyl alcohol (95%, 30 cc) and treated with a solution of sodium periodate (150 mg) in sulphuric acid (6 cc). The mixture was kept for 18 hours at room temperature and then poured into excess of water (150 cc). The white precipitate so obtained was extracted a number of times with ether. The combined ethereal solution was washed with water and the solvent recovered. The colourless residue left over was crystallised from methanol as colourless needles m.p. 223-24°. The product was identified as the methyl ester, the starting material by mixed melting point.

Attempted preparation of isopropylidene derivative of the methyl ester:

The methyl ester (500 mg) in dry acetone (25 cc) was treated with a drop of concentrated hydrochloric acid. No precipitate separated out; and hence the solvent was removed and the product crystallised from methanol, m.p. 223-24°. It showed no depression on melting with a sample of methyl ester of acacic acid.

Hydrogenation of the diacetyl lactone:

The diacetyl lactone (100 mg) was hydrogenated in acetic acid in presence of Adam's platinum oxide catalyst at atmospheric pressure. It crystallised from methanol in the form of plates, m.p. 280-85°. It did not give a colour with tetranitromethane.

Analysis:

Found	C 72.73%	H 9.78%
	C 72.5%	H 9.90%
Calc.for $C_{34}H_{52}O_6$	C 73.34%	H 9.41%

Partial hydrolysis of the diacetyl lactone:

The lactone (500 mg) was refluxed for 40 minutes with potassium carbonate in methanol (200 cc), dioxane (20 cc), and water (20 cc). The volume was reduced under suction, the solution poured into excess of water, the product extracted

with ether, dried and the ether recovered. The product was chromatographed on acid washed alumina with no improvement in the melting point.

Acid hydrolysis of the diacetyl lactone:

The diacetyl lactone (500 mg) was dissolved in methanol (50 cc) containing hydrochloric acid (10 cc) and the mixture refluxed for two hours. It was then concentrated to half the volume under reduced pressure, the solution poured into water and the substance extracted with ether. The ether was washed with water till free of acid dried over anhydrous sodium sulphate and recovered. The substance was crystallised from methanol, m.p. 252-54°. It gave a positive colour with tetranitromethane.

Acetylation of the acid hydrolysis product of the lactone:

The acid hydrolysis product (200 mg) was acetylated in the cold with acetic anhydride (10 cc) and pyridine (15 cc). The product was worked out in the usual manner and crystallised from methanol, m.p. 234-36°. It showed no depression on a mixed melt with a sample of acacic acid diacetyl lactone.

Oxidation of the acid hydrolysis product:

The acid hydrolysis product (0.2 g) was dissolved in anhydrous pyridine (4 cc) and to it was added a previously prepared mixture of chromium trioxide (0.3 g) in pyridine (8 cc).

It was kept at room temperature for eighteen hours and then diluted with water. The product was extracted with ether and washed well with dilute acid, water and dried over anhydrous sodium sulphate. Recovery of the ether furnished the substance which was crystallised from methanol:chloroform; m.p. 338-42°. It gave a positive colour with tetranitromethane.

The product on thin layer chromatography on alumina using the system cyclohexane-ethyl acetate showed two spots, a major spot and another spot with a faster running rate in traces.

Preparation of semicarbazone of the ketone:

Semicarbazone hydrochloride (1.1 g) and anhydrous sodium acetate (1.6 g) were dissolved in a small amount of water and alcohol (10 volumes) added to it. The precipitated sodium chloride was filtered off. To an excess of this solution an alcoholic solution of the compound (0.1 g) was added. Warmed the mixture gently for 10 minutes and on standing a solid separated. This was collected and crystallised from water: alcohol; m.p. 278-82°.

Wolff Kishner reduction of the ketone:

The ketone (100 mg) was dissolved in diethyleneglycol (20.3 cc) containing metallic sodium (400 mg) and to it was

added hydrazine hydrate (100%:freshly distilled on equal amount of sodium hydroxide) till the reaction mixture boiled at 180°. It was refluxed at this temperature for eighteen hours when the condenser was removed and the hydrazine hydrate allowed to evaporate till the mixture boiled at 210°. The condenser was then replaced and the refluxing continued for another twentyfour hours. The reaction mixture was then cooled to room temperature, diluted with water and extracted with ether a number of times. The alkaline aqueous solution on acidification with hydrochloric acid gave no acid product. The combined ethereal layer was washed with water to remove the alkali, dried and recovered. The colourless solid residue obtained was crystallised from methanol:ethyl acetate, in colourless needles m.p. 185-88°. It gave a positive reaction with tetranitromethane.

Analysis:

Found	C	84.20%	H	11.73%
Calc. for $C_{29}H_{48}O$	C	84.40%	H	11.72%

Acetylation of the Wolff-Kishner reduction product:

The substance (60 mg) was acetylated with pyridine and acetic anhydride in the usual manner. The acetate crystallised from ethyl acetate:methanol in plates, m.p. 210-16°. It gave a positive colour with tetranitromethane.

Dehydration of the acetyl methyl ester:

The acetyl methyl ester (300 mg) was dissolved in pyridine (10 cc) and phosphorous oxychloride (2 cc) added to it. The solution was refluxed for five hours. It was then allowed to cool and diluted with ice cold water. The product was extracted with ether and the ether washed free of acid with water and dried. Evaporation of the ether furnished the dehydration product. It crystallised from methanol as shining needles, m.p. 280-82°. It gave dark colour with tetranitromethane.

Analysis:

Found:	C	73.54	H	9.65
	C	73.78	H	9.19
Calc. for $C_{35}H_{52}O_6$	C	73.91	H	9.22

Hydrogenation of the dehydration product of acetyl methyl ester:

The dehydration product (200 mg) was dissolved in glacial acetic acid (53 cc) and hydrogenated at atmospheric pressure in presence of Adam's platinum oxide catalyst (400 mg). No more hydrogen was absorbed after four hours. The catalyst was filtered off and the substance recovered by evaporating the solution to dryness under reduced pressure. The product crystallised in the form of shining needles,

m.p. 256-70°. It gave a positive colour with tetranitromethane. It showed no depression on melting with an authentic sample of acetyl methyl ester of proceric acid.[203]

Analysis:

Found:	C 72.88%	H 9.24%
	C 72.76%	H 9.15%
Calc. for $C_{35}H_{54}O_6$	C 73.64%	H 9.54%

2. Study of the seeds of Albizzia amara Benth:

Defatting:

The finely powdered seeds (425 g) were repeatedly extracted with light petroleum ether (40-60). The recovery of the solvent gave the oil (30 g).

Extraction of the saponin:

The defatted seed powder was dried and extracted with ethanol four times. The recovery of ethanol yielded a dark coloured syrup. This was successively extracted with ether, petroleum ether, carbontetrachloride, chloroform and acetone. The residual semi solid was then dissolved in the minimum quantity of alcohol and the saponin precipitated by addition to a large volume of ether. The ether was decanted off from the precipitated saponin (53 g).

Hydrolysis of the saponin:

The saponin (53 g) was dissolved in a large amount of water and heated with sulphuric acid (10%) on a water bath for one hour and then refluxed for another hour. The sapogenin formed was filtered, washed free of acid and dried (22 g).

Separation of the acid and neutral fractions:

The crude genin (22 g) was refluxed with alcoholic potassium hydroxide (10%; 700 cc) for an hour. About half of the solvent was recovered and the contents diluted with about two litres of water. It was extracted with ether several times, the ethereal layers combined, washed free of alkali and the ether recovered to yield a small amount of neutral portion.

The aqueous layer was treated with hydrochloric acid which precipitated the acid genin. It was filtered washed free of acid and dried (10.5 g).

Acetylation of the acid genin:

The crude acid genin (3 g) was dissolved in pyridine (50 cc) and acetic anhydride (30 cc) added to it. The mixture was kept overnight at room temperature. The acetate was then precipitated by dropwise addition of the mixture to ice cold water with constant stirring. The acetate was filtered, washed free of pyridine and dried. Repeated

crystallisations from methanol furnished the acetate (0.8 g), m.p. 255-60°. It gave a positive colour with tetranitromethane.

Preparation of the acetyl methyl ester:

The acetate (0.2 g) was dissolved in ether (50 cc) and an excess of ethereal solution of diazomethane added to it. The mixture was kept at room temperature overnight and the excess of diazomethane and ether evaporated off on a water bath. The colourless residue left over was crystallised from methanol (0.18 g), m.p. 201-2°. Mixed melting point with acetyl methyl echinocystate 200-201°. (Cf: m.p. of acetyl methyl echinocystate 200-201°).

3. Study of the seeds of Pithecolobium dulce Benth (Inga dulcis Willd).

Defatting:

Well powdered seeds (960 g) were extracted with light petroleum ether (40-60) thrice. The recovery of the solvent left the oil (89 g). The exhausted seeds were dried before the next operation.

Extraction:

The defatted seeds were extracted with alcohol (5 1) four times and the combined extracts evaporated to yield a gummy mass. It was successively treated with ether, petroleum

ether, carbontetrachloride and acetone. The residue was dissolved in the minimum quantity of alcohol and precipitated by addition to a large volume of ether acetone, several times.

Hydrolysis of the saponin:

The saponin was dissolved in water (3.5l) and hydrolysed with sulphuric acid (350 g) by heating the solution first on a water bath for one hour followed by refluxing the solution for another hour. The sapogenin formed was filtered, washed free of acid and dried (7.8 g).

Separation of the acid and neutral portions:

The genin was dissolved in alcohol (250 cc) containing sodium hydroxide (25 g) and refluxed for half hour and then the volume concentrated to about half. The solution was then poured into water and extracted with ether. Recovery of the ether solution yielded the neutral portion.

The aqueous layer was acidified with hydrochloric acid and the precipitated genin filtered, washed free of acid and dried. (6.25 g).

Acetylation of the acid genin:

The acid genin was dissolved in pyridine (25 cc) and acetic anhydride (25 cc) added to it. The mixture was kept overnight and then poured into crushed ice, the solid obtained

filtered, washed free of acid and pyridine, dried, taken up in methanol and charcoaled. On repeated crystallisation from methanol it separated into two fractions A, m.p. 286-88°. (Cf. proceric acid acetate m.p. 288-90°) and B, 215-20°.

Methylation of A:

The product was dissolved in ether and an ethereal solution of diazomethane added to it. The mixture was kept overnight ether evaporated and residue crystallised from methanol as needles m.p. 269-74° (Cf. Acetate methyl ester of proceric acid m.p. 268-70°) Mixed m.p. with methyl procerate acetate 270-74°).

Methylation of B:

The methyl ester of B was prepared in the above manner and it crystallised from methanol, m.p. 217-22°. It gave a positive colour with tetranitromethane. It also gave a positive reaction with the stannic chloride reagent.

4. Study of the seeds of Sesbania speciosa Taub:

Defatting:

The finely powdered seeds (1120 g) were extracted with light petroleum ether thrice. The recovery of the solvent gave the oil (24 g).

Extraction of the saponin:

The defatted seed powder was dried and then extracted with ethanol four times. The combined extracts were

concentrated to yield a thick syrup. The syrup was then successively treated with ether, petroleum ether, carbon-tetrachloride and acetone. The residue left over was dissolved in the minimum quantity of ethanol and the saponin precipitated by addition of the solution to a large quantity of ether. The ether was decanted off to yield the saponin (39.5 g).

Hydrolysis of the saponin:

The saponin (39.5 g) was dissolved in a large quantity of water and heated with sulphuric acid (10%) on a water bath for one hour and then refluxed for another hour. The sapogenin formed was filtered, washed free of acid and dried (6.2 g).

Separation of the acid and neutral portions:

The crude genin (6.2 g) was dissolved in alcohol (500 cc) containing sodium hydroxide (30 g) and refluxed for two hours. About half of the solvent was recovered and the solution diluted with water. It was then repeatedly extracted with ether. The recovery of the combined neutral extracts furnished the neutral portion (1.1 g).

The alkaline aqueous layer was treated with hydrochloric acid which precipitated the acid genin. It was filtered washed free of acid and dried (3.6 g).

Acetylation of the acid genin:

The crude acid genin (1 g) was dissolved in pyridine (25 cc) and acetic anhydride (15 cc) added to it. It was heated on a boiling water bath for two hours, cooled and poured into ice cold water, drop by drop with constant stirring. The precipitated acetate was filtered and washed free of pyridine and dried. Repeated crystallisations from methanol gave the acetate, m.p. 260-64°. Mixed melting point with oleanolic acid acetate showed no depression.

Preparation of the methyl ester:

The crude acid genin (1 g) was dissolved in ether (100 cc) and excess of an ethereal solution of diazomethane added to it. After keeping it at room temperature for four hours, the excess of ether and diazomethane were evaporated off and the residue crystallised from methanol repeatedly, m.p. 196-98°. Mixed melting point with oleanolic acid methyl ester showed no depression. It gave a positive colour with tetranitromethane.

Examination of the neutral fraction:

Repeated attempts to crystallise the neutral portion were unsuccessful and hence it was chromatographed.

Chromatography of the neutral portion:

The neutral portion (1 g) was chromatographed on alumina In all twenty nine fractions were collected, using different solvents.

Fractions	Products
1-4 Benzene	Oil
5-9 Benzene	Product I
10-12 Benzene	No elution
13-14 Benzene-acetone (3:1)	Traces
15-20 benzene-acetone (3:1)	Product II
21-24 Benzene:acetone (1:1)	Nil
25-27 Acetone	Nil
27-29 Alcohol	Nil

In the initial stages fractions of 30 cc each were collected and in the final stages fractions of 75 cc.

Examination of product I:

The combined material from fractions 5-9 was crystallised from methanol. It crystallised in the form of plates, m.p. 145-50°. It gave no colour with tetranitromethane. It showed no depression on mixed melting point with an authentic sample of β -sitostanol.

Acetylation of product I:

The product I (50 mg) was acetylated in the cold and

worked out in the usual manner. It crystallised from methanol in the form of plates, m.p. 132-36°. It showed no depression on mixed melting point with an authentic sample of β-sitostanol acetate. It gave no colour with tetranitromethane.

Examination of the product II:

The fractions 15-20 were combined and the substance crystallised from methanol, m.p. 252-58°. It gave a colour with tetranitromethane. It also gave a positive colour reaction for triterpenes with the stannic chloride reagent.

Acetylation of the product II:

The product II (50 mg) was acetylated with pyridine and acetic anhydride in the cold and worked out in the usual manner. It crystallised from methanol in clusters, m.p. 159-64°. It gave a positive colour with tetranitromethane and with stannic chloride reagent.

5. Study of the leaves of Psidium guyava Linn:

Extraction:

The leaves (790 g) were twice extracted in cold with ether (4 litres). The extracts were combined and recovered on a water bath to give a greenish gummy mass.

Separation of the acid and neutral portions:

The greenish mass was refluxed with alcoholic sodium hydroxide (5%) for two hours and the volume reduced to half on a water bath. The solution was poured into a large amount of water and extracted a number of times with ether. The ethereal layers were combined, washed free of alkali and the ether recovered on a water bath to yield the neutral fraction. All attempts to crystallise this product from various solvents were unsuccessful and hence it was chromatographed.

Chromatography of the neutral fraction:

The neutral portion (2 g) was chromatographed on alumina (50 g). Elution with benzene (13 fractions) was followed by elution with benzene:acetone mixture with progressively increasing quantities of acetone. In all eighty fractions of 30 cc each were collected. Each fraction was evaporated to dryness and residue allowed to crystallise from methanol.

Product m.p. 80-82:

Fractions 1-4 gave oil in admixture with some solid product. The oil was removed by treatment with cold methanol and the solid obtained was crystallised from benzene: petroleum ether to give colourless crystals; m.p. 80-82^{0}.

Isolation of β-sitosterol:

Fractions 5-8 were combined and crystallised from methanol to give colourless plates m.p. 137-39°. It did not depress the melting point of an authentic sample of β-sitosterol.

Acetylation of β-sitosterol:

The product m.p. 137-39° was acetylated with acetic anhydride and pyridine in the cold and the reaction poured into iced water after eighteen hours. The acetate obtained was filtered and washed well with water and crystallised from methanol as colourless needles m.p. 128-24°.

Analysis:

Found:	C 81.25%	H 11.56%
Calc. for $C_{31}H_{52}O_2$	C 81.57%	H 11.40%

Product m.p. 153-55°

Elution of the chromatogram with benzene containing 5% to 40% acetone brought forth no product but with 50% benzene:acetone a product was eluted which on crystallisation from methanol had m.p. 153.55°. It gave a positive reaction with tetranitromethane and with the stannic chloride reagent.

Study of the acid product:

The alkaline aqueous layer after ether extraction was freed of dissolved ether on a water bath and cooled.

Acidification with hydrochloric acid precipitated the acid product. It was filtered washed free of acid and dried.

Preparation of the acetate:

The acid was acetylated with pyridine and acetic anhydride in the cold in the usual manner. Crystallisation from methanol gave a product m.p. 226-31°. It gave a positive colour with tetranitromethane.

Preparation of the methyl ester:

An ethereal solution of the acid genin was treated with an excess of ethereal solution of diazomethane and kept overnight. The excess ether and diazomethane was evaporated off. On crystallisation from methanol it melted at 168-72°.

Preparation of the acetyl methyl ester:

The methyl ester was acetylated in the cold with acetic anhydride and pyridine. On crystallisation from methanol it melted at 236-39°. It gave a positive colour with tetranitromethane.

Chromatography of the methyl ester:

The methyl ester (2.3 g) was chromatographed on alumina (70 g). Altogether forty fractions were collected. Three products were isolated, termed as product I, product II and product III.

Product I:

The fractions 5-8 (benzene)were combined and evaporated. The residue was then crystallised from methanol, m.p. 102-104°. It showed a positive colour with tetranitromethane.

Product II:

The fractions 22-24 (ether:benzene; 1:1) were evaporated and the residue crystallised from methanol, m.p. 212-14°. As this fraction seemed to be a mixture it was again chromatographed on alumina. Twentyfive fractions were collected. The fractions 13-16 (ether:benzene; 1:1) were combined and the product crystallised from methanol, m.p. 205-207°. Mixed melting point with an authentic sample of methyl guaijavolate, 209-211° (Cf: methyl guaijavolate m.p. 210-11°).

The fractions 23-25 were combined and the product crystallised from methanol, m.p. 220-22°.

Product III:

The fractions 26-30 were combined and crystallised from methanol, m.p. 220-22°. This product was found to be identical with the product m.p. 220-22° isolated by the rechromatography of the product II by mixed melting point. It gave a positive colour with tetranitromethane.

6. Study of the seed kernels of Balanites roxburghii Planch:

Defatting:

Well powdered seed kernels (394 g) were exhausted in a soxhlet apparatus with light petroleum ether (40-60). The recovery of the solvent gave an golden yellow oil (137 g).

Extraction:

The defatted seed powder was exhausted with 95% alcohol in soxhlet apparatus and the solvent recovered when a brown syrupy liquid was left over. The syrup was dissolved in ethyl alcohol and filtered. The solvent was recovered and the residue extracted successively with ether, petroleum ether, chloroform, carbontetrachloride and acetone. The residue was dissolved in alcohol and added dropwise to a large volume of ether/acetone when a light brown precipitate was obtained. This operation of dissolution and precipitation was repeated a number of times and later the saponin decolourised with activated charcoal. This gave a colourless powder m.p. 128-36^{0} giving all the tests for saponin.

Electrophoresis:

The saponin was put on a strip of filter paper Arches No. 302 and horizontal electrophoresis done using borate buffer (sodium tetraborate 9.54 g/1). After drying the

paper in infrared rays it was sprayed with Sannie's cinnamic aldehyde reagent and then heated in an oven at 100°. It showed that the saponin is a mixture, as three spots were obtained, one major and two minor. The electrophoresis was done at 310 volts for eight hours.

Paper chromatography:

The saponin was chromatographed on Whatman filter paper No. 1 using both ascending and descending techniques and butanol:acetic acid:water (4:1:5) as solvent mixture. The chromatogram was air dried and then sprayed with Sannie's cinnamic aldehyde reagent as well as with p-dimethyl-aminobenzaldehyde reagent. It was dried in an oven at 105° for five minutes. It also showed three spots.

Treatment of crude genin with Girard's reagent P:

The crude genin (700 mg) was dissolved in alcohol (20 cc) containing acetic acid (10%) and heated for one hour. The solution was diluted with water (160 cc) containing caustic soda (1.26 g). The solution was extracted with ether a number of times. The recovery of ether after washing gave the non-ketonic genin (650 mg).

The solution left out after ether extraction was decomposed by addition of hydrochloric acid (7.6 gc). After keeping overnight it was extracted with ether and the

recovery of the ether gave the ketonic part (10 mg). It could not be crystallised.

Acetylation of the ketonic genin:

The ketonic part obtained was acetylated with pyridine and acetic anhydride in hot on a water bath. The acetate was poured in ice water mixture and the acetate filtered. It could not be crystallised from any solvent.

Chromatography of ketonic acetate on filter paper:

The acetate of the ketonic genin was chromatographed on Whatman filter paper No. 1 using ascending technique and petroleum ether (40-60); ether:water (400:30:70) as solvent system as well as petroleum ether:toluene:ethanol:water (400:50:10:90) alongside with an authentic sample of krypto-genin acetate and diosgenin acetate. The chromatogram was developed by spraying with Sammie's cinnamic aldehyde reagent. The product isolated was quite different from these two authentic samples in the position of the spot and in its colour.

Purification of the saponin:

The saponin m.p. 128-36^{0} was applied to a prewashed filter paper Whatman No. 3 in the form of streaks and then the chromatogram run for fourteen hours using the descending technique and butanol:acetic acid:water (4:1:5) as solvent mixture. From the filter paper after drying in the air,

test strips were cut and developed by spraying with Sannié's cinnamic aldehyde reagent. The corresponding relevant portions were marked and the strips containing the major saponin were cut out and the saponin eluted by boiling alcohol (70%). The alcoholic extract of the saponin was concentrated and then the saponin solution was added to a large amount of acetone which precipitated the saponin. It was f ltered and reprecipitated with acetone. The saponin thus obtained had m.p. 132-36°.

Rechromatography of the saponin:

The saponin obtained by paper chromatography was rechromatographed on Whatman filter paper No.1 in the same manner as described earlier. The chromatogram after development showed only one spot this time, confirming that the saponin is now pure.

Isolation of the sapogenin:

The saponin m.p. 132-36° was dissolved in large amount of water and hydrolysed with sulphuric acid (10%) by heating the solution first on boiling water bath for one hour and then completing the hydrolysis by refluxing the solution for two hours more. After completion of the hydrolysis the precipitate of the genin obtained was filtered and washed free of acid. The precipitate was refluxed with alcoholic potash solution

(10%) for one hour. Then half of the solution was distilled off and the solution diluted with a large amount of water (2 1) and extracted with ether thrice. The ethereal extracts were combined and washed with water till they were free of alkali. The ether was recovered and it left a neutral sapogenin which was crystallised from acetone. On recrystallisation from acetone it gave colourless needles, m.p. 196-98°. The mixed melting point with authentic diosgenin was 196-98°.

Acetylation of the sapogenin:

The genin was acetylated with aceticanhydride and pyridine in the cold. The reaction mixture was poured into the ice water after eighteen hours and the acetate obtained was filtered. It was washed well to remove the pyridine and then crystallised from methanol:ethyl acetate (1:1) in colourless needles m.p. 201-203°. It gave a positive reaction with tetranitromethane. The melting point with an authentic sample of diosgenin acetate was undepressed.

Benzoylation of the genin:

The genin was treated with freshly distilled benzoyl chloride in the cold in pyridine solution. The solution after twentyfour hours was poured in dilute sulphuric acid. It was kept overnight and the precipitate obtained was

filtered and washed free of pyridine. It was crystallised from methanol as colourless crystals m.p. 233-36°. (Cf: diosgenin benzoate m.p. 236-41°).

Isolation of the sugars:

The sulphuric acid hydrolysate obtained after hydrolysing the saponin was neutralised with freshly precipitated barium carbonate. The precipitate of the barium sulphate obtained was filtered and washed with hot water and filtrates and the washings were combined and evaporated to dryness in a vacuum oven at 35-40°. It gave a light brown syrup of the sugar. The syrup was dissolved in a small amount of water and filtered again to remove any barium sulphate left out. The filtrate was dried in the vacuum to yield a syrup of the sugars.

Paper chromatography of the sugars:

The syrupy residue of the sugars was dissolved in a few drops of water and chromatographed on Whatman filter paper No. 1 along with authentic sugars using the solvent mixture, butanol:ethanol:water (4:11:19) and utilising the descending technique. The spots were revealed by spraying aniline hydrogen phthalate solution. The presence of three sugars, glucose, xylose and rhamnose was noted. The absence of ketohexoses was confirmed by spraying the chromatogram with urea-HCl reagent when no spots were observed.

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